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Factor Analysis in Discriminating the Racial Origin of Sockeye Salmon (*Oncorhynchus nerka*)

H. B. MESSINGER[1] AND H. T. BILTON

Fisheries Research Board of Canada
Pacific Biological Station, Nanaimo, B.C.

MESSINGER, H. B., AND H. T. BILTON. 1974. Factor analysis in discriminating the racial origin of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 31: 1–10.

A comparison was made between the procedure of factoring variables before using them in discriminant analysis and the usual procedure of using the original variables in discriminant analysis. The results indicated factoring seven scale measurements on sockeye salmon (*Oncorhynchus nerka*) with varimax rotation produced four new variables which gave more valid results in classifying sockeye salmon by area of origin than the original variables when discriminant functions were computed. Although the results on the basic data from which the functions were derived were not as good using the four factor scores as the seven original variables, the accuracy of classification was much more consistent in the test data with the factor scores. The loss in accuracy was at least 2½ times as great for functions based on the original variables as for ones based on factor scores. The errors in classifying fish to their individual places of origin were perhaps too large for the procedure to be useful in the field, but the accuracy of classification to the British Columbia or Alaska region was quite high.

MESSINGER, H. B., AND H. T. BILTON. 1974. Factor analysis in discriminating the racial origin of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 31: 1–10.

Les auteurs comparent la procédure d'analyse factorielle des variables avant leur utilisation dans l'analyse discriminante avec la méthode ordinaire suivant laquelle les variables originelles sont utilisées dans l'analyse discriminante. Les résultats démontrent que l'analyse factorielle de sept mesures effectuées sur des écailles de saumon nerka (*Oncorhynchus nerka*) par rotation varimax produit quatre nouvelles variables, qui donnent des résultats plus valides que les variables originales utilisées dans l'analyse discriminante lorsqu'il s'agit de classifier les saumons suivant leur lieu d'origine. Bien que les résultats obtenus à partir des données de base dont les fonctions ont été dérivées ne soient pas aussi bonnes quand on utilise les quatre nouvelles variables que quand on se sert des sept variables originelles, la classification

[1]Present address: Ministry of State for Urban Affairs, Ottawa, Ont.

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se fait avec une précision beaucoup plus uniforme lorsque les quatre nouvelles variables sont utilisées. De fait, il y a perte de précision d'au moins 2½ fois pour les fonctions basées sur les variables originelles, comparativement à celles basées sur les variables nouvelles. En général, les erreurs, lorsqu'il s'agit de classifier les poissons suivant le lieu d'origine individuel, sont peut-être trop grandes pour que la méthode soit utile sur le terrain. Par contre, la séparation entre les saumons de la Colombie-Britannique et ceux de l'Alaska se fait avec une assez grande précision.

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ADAM and Enke (1970) have suggested factoring variables before using them in discriminant analysis. In this study, a comparison is made between this procedure and the usual one of not factoring. Arguments against factoring could be the possible loss of discriminating information in reducing the number of variables (from 7 to 4 in the present case) and the additional computing involved. The advantages could be: loss of information is relatively small considering how much the complexity of the data is reduced; the factors (if rotated to simple structure) may be interpretable, and since they can be made orthogonal, the coefficients of the discriminant functions may be more reliable guides to the relative importance of the "variables" (now the factor scores) than is usually the case; and the factor scores are more apt to be normally distributed than the original variables.

The data on sockeye salmon (*Oncorhynchus nerka*) have been described by Bilton (1970) and the discriminant analysis without factoring by H. T. Bilton and H. B. Messinger (unpublished data). Thus, we shall provide here only a brief summary. The results may be compared with those of Bilton (1971) and Amos et al. (1963) on pink salmon.

The results of the earlier discriminant analysis, using seven of the original variables, indicated that quite large proportions of almost all of the major runs of age 1.2 and 1.3[2] fish from the British Columbia and Alaska regions could be correctly classified to their region of origin. Thus, high proportions of fish caught on the high seas of unknown origin could be classified to region of origin on the basis of their scale characters. However, the accuracy or classification by run varied so much among the runs that the procedure cannot be recommended for field use unless one was interested only in those runs which can be identified well, such as Rivers and Smith inlets together, and Bristol Bay.

Materials and Methods

Samples of 20 sockeye of each age from each area (for age 1.2 and 1.3 fish there were 11 and eight areas, respectively) were chosen randomly from each of 4 years for use in developing the discriminant functions. An additional 50 samples from each area, when available, were chosen randomly from each of the same years to provide test samples. Preliminary studies were made on data for a single age (1.2) and year (1964), and final

[2]The European system of age designation is used in this report. The first digit indicates the number of annuli formed in fresh water and the second digit indicates the number of annuli formed while the fish was in the ocean.

TABLE 1. Multivariate analysis of variance: significance test on basic samples (F-tests) of age 1.2 and 1.3 sockeye salmon (*Oncorhynchus nerka*) (NS — no significant difference; ** — significant difference at the 0.01% level).

	Age 1.2		Age 1.3	
Data used	Original variables	Factor scores	Original variables	Factor scores
All years				
H_1	3.58 (**)	1.98 (**)	3.42 (**)	1.16 NS
H_2	21.08 (**)	32.20 (**)	25.45 (**)	38.82 (**)
Range of D values	210–10^{14}	0.004–0.365	1,881–295,495	0.018–0.229
$\eta^2 = 1-\Lambda$	0.8969	0.8546	0.8936	0.8421
1964 only				
H_1	1.99 (**)	1.64 (**)	1.98 (**)	1.70 (**)
H_2	10.90 (**)	15.61 (**)	10.49 (**)	11.68 (**)
Range of D values	380–33,368	0.0126–0.203	96–394,821	0.0042–0.257
$\eta^2 = 1-\Lambda$	0.9384	0.8864	0.9281	0.8184

studies for the years 1961–64 and ages 1.2 and 1.3 (as data were available for the various runs). The former also provided a comparison of the results within a single year versus a span of 4 years.

Discriminant analysis is based on assumptions that the dispersions of the various groups are equal and that the vectors of group means are not equal. The former hypothesis is H_1 and the latter H_2, in the terminology of Cooley and Lohnes (1962). Their computer program, adapted to our machine, was used to test these assumptions.

The original variables were:

A_1 — width of freshwater zone from the scale focus to the freshwater annulus.

A_2 — width of the first ocean zone from the last freshwater circulus to the first ocean annulus.

A_3 — width of the second ocean zone from the first ocean annulus to the second ocean annulus.

A_4 — width of the third ocean zone from the second ocean annulus to the third ocean annulus.

C_1 — the number of circuli in the freshwater zone from the scale focus to the freshwater annulus.

C_2 — the number of circuli in the first ocean zone from the last freshwater circulus to the first ocean annulus.

C_3 — the number of circuli in the second ocean zone from the first ocean annulus to the second ocean annulus.

C_4 — the number of circuli in the third ocean zone from the second ocean annulus to the third ocean annulus.

A_2/C_2 — the ratio of the width of the first ocean zone divided by the number of circuli in the first ocean zone.

C_2/C_1 — the ratio of the number of circuli in the first ocean zone divided by the number of circuli in the freshwater zone.

A_4 and C_4 were dropped as inappropriate for age 1.2 an for age 1.3 fish as there appeared to be little difference in these characters among the various runs. C_2/C_1 was omitted on the grounds of (a) redundancy — it is correlated strongly in most groups with C_2, C_1, and A_1; and (b) inconsistency — its standard deviation varied more among groups (from 0.37 to 1.10) than any other variable and similarly for its correlations (with C_3 the range was from −0.72 to +0.68).

The factor analytic procedure used was that of principal components with varimax rotation. Factor scores were computed by the regression method for input to the multiple-group discriminant analysis program (after the method of Cooley and Lohnes 1962, as implemented by Thomson and Calaprice 1970) after being used to check for outliers. Since some fish caught in one area may in fact be from another area, an ad hoc procedure was used to detect extremely atypical fish in the samples used to derive the discriminant functions.

For each area, the average score on the first four factors was used to define a hypothetical subject. The Euclidean distance of each of the other subjects in the same group from that subject was computed. An arbitrary value was selected after inspecting the various runs and cases falling above this were rejected. To help estimate a reasonable value, the differences between the minimum and mean distances observed in each group were computed[3]. Arguing from the symmetry expected in the factor score distributions, there should be no observations greater than the mean plus this difference. The observed differences ranged from 0.51 to 1.40. The critical value used was 1.5. When a subject was rejected, it was replaced from the pool of test subjects, the distances were recomputed, and so on until no further rejections occurred. In age-group 1.2, 26 out of 540 subjects were rejected and in age-group 1.3, 15 out of 456. The largest number (seven) of rejections occurred in Area 9 in age 1.2; this area is known to mix with Area 10. Area 65 had 11 rejections for the two ages combined. The rather large number of rejections which occurred in Area 65 (Cook Inlet) may have been associated with the fact that sockeye from Fish Creek, one of the runs from this area, have quite distinctive scale patterns. Scale of sockeye from this run have on the average a higher number of circuli in the freshwater zone of their scales than for other runs from this area.

[3]A referee has suggested that a better method would be to use the Mahalanobis distance of each case from the centroid of all cases in the same group as approximately distributed as chi-square, with 4 df since here there were four factors.

Results

The interrelationships among the seven variables are the basis for computing discriminant functions and factor scores. The observed correlation matrix was as follows:

The upper right triangle contains the figures for the age 1.2 group and the lower left for the age 1.3 group.

	A_1	A_2	A_3	C_1	C_2	C_3	A_2/C_2
A_1	–	0.0565	−0.3066	0.9096	−0.4064	−0.3524	0.4928
A_2	−0.1705	–	−0.1486	0.0189	0.5824	−0.2388	0.4035
A_3	−0.3257	−0.2183	–	−0.2622	0.2620	0.8343	−0.4377
C_1	0.9188	−0.2079	−0.3254	–	−0.3263	−0.2588	0.3606
C_2	−0.5615	0.5301	0.2174	−0.5031	–	0.3342	−0.4922
C_3	−0.3093	−0.2705	0.8553	−0.2785	0.2839	–	−0.6167
A_2/C_2	0.4594	0.2972	−0.4320	0.3653	−0.6292	−0.5561	–

TABLE 2. Comparison of classification of age 1.2 and 1.3 sockeye salmon by discriminant functions with true origin, 1961–64 basic data, using original variables and factor scores.

		B.C. region				Alaska region							
		Area assigned											
	True area	29	9	10	4	61	64	65	66	67	68	70	Total
						Original variables, age 1.2							
B.C. region	29	25	4	3	12	2	2	5	7	0	0	0	60
	9	2	67	6	4	1	0	0	0	0	0	0	80
	10	3	40	24	9	1	0	0	0	0	1	2	80
	4	2	5	1	60	1	1	3	2	4	0	1	80
Alaska region	61	1	2	0	1	15	0	0	1	0	0	0	20
	64	0	0	0	0	0	16	2	1	0	0	1	20
	65	0	0	0	7	1	2	17	2	1	1	9	40
	66	2	0	0	7	1	0	4	19	4	2	8	47
	67	0	0	0	1	0	0	2	0	15	0	2	20
	68	0	0	0	0	3	0	0	2	1	11	3	20
	70	2	0	0	3	0	0	6	4	3	3	59	80
													547
						Factor scores, age 1.2							
B.C. region	29	14	4	5	21	3	2	5	2	2	0	2	60
	9	2	61	11	4	1	0	1	0	0	0	0	80
	10	2	39	23	10	3	0	1	0	0	0	2	80
	4	4	5	5	52	1	1	4	2	4	0	2	80
Alaska region	61	0	0	4	4	9	0	1	2	0	0	0	20
	64	2	0	0	0	0	14	1	1	0	0	2	20
	65	2	0	0	8	2	0	12	1	1	1	13	40
	66	1	0	1	8	0	0	3	15	6	2	11	47
	67	0	0	0	3	0	0	1	1	11	0	4	20
	68	0	0	0	1	1	0	0	5	1	7	5	20
	70	1	0	0	5	0	1	6	3	1	5	58	80
													547
			9	10	4	61	65	66		67	70		Total
						Original variables, age 1.3							
B.C. region	9		63	12	4	0	0	1		0	0		80
	10		30	36	8	2	3	0		0	1		80
	4		1	6	61	0	5	3		2	2		80
Alaska region	61		4	3	2	17	3	5		4	1		39
	65		1	1	9	1	19	3		0	6		40
	66		2	0	3	0	1	20		8	3		37
	67		0	0	1	1	0	4		11	3		20
	70		0	0	7	0	8	4		4	57		80
													456
						Factor scores, age 1.3							
B.C. region	9		56	18	3	2	0	0		0	1		80
	10		37	29	10	2	1	0		0	1		80
	4		0	4	64	4	3	1		1	3		80
Alaska region	61		7	2	3	20	1	0		6	0		39
	65		1	2	12	3	9	2		2	9		40
	66		1	1	7	6	2	10		6	4		37
	67		0	0	2	4	0	3		5	6		20
	70		0	0	9	0	2	3		2	64		80
													456

TABLE 3. Comparison of classification of age 1.2 and 1.3 sockeye salmon by discriminant functions with true origin, 1961–64 test data, using original variables and factor scores.

		B.C. region				Alaska region							
		Area assigned											
	True area	29	9	10	4	61	64	65	66	67	68	70	Total
						Original variables, age 1.2							
B.C. region	29	45	14	11	45	3	5	12	4	4	2	5	150
	9	9	121	29	6	2	1	2	0	0	0	1	171
	10	11	110	27	17	3	2	8	2	1	0	0	181
	4	25	6	5	91	0	1	12	5	12	0	3	160
Alaska region	61	4	7	4	12	10	3	2	5	0	2	1	50
	64	3	0	0	6	4	24	8	0	0	0	5	50
	65	11	1	2	5	0	7	40	2	2	4	26	100
	66	1	0	0	0	0	0	2	4	0	0	0	7
	67	0	0	1	3	0	0	0	1	4	0	8	17
	68	2	2	1	2	0	2	0	10	1	14	16	50
	70	2	0	1	9	0	8	20	12	7	3	138	200
													1136
						Factor scores, age 1.2							
B.C. region	29	42	15	10	55	6	3	6	3	4	0	6	150
	9	8	120	28	5	3	1	3	0	0	1	2	171
	10	11	103	31	22	6	1	2	0	2	0	3	181
	4	22	5	4	101	1	0	7	3	11	0	6	160
Alaska region	61	5	7	5	11	8	1	4	7	0	0	2	50
	64	7	0	0	5	1	26	6	1	0	0	4	50
	65	15	1	1	9	1	5	26	4	4	4	30	100
	66	1	0	0	1	0	0	0	2	1	0	2	7
	67	0	0	0	3	0	0	1	1	3	2	7	17
	68	1	1	1	1	2	0	1	9	0	20	14	50
	70	0	0	0	10	0	2	14	8	8	3	155	200
													1136
			9	10	4	61	65	66	67	70			Total
						Original variables, age 1.3							
B.C. region	9		109	63	12	3	5	1	0	0			193
	10		95	72	15	1	4	2	0	1			190
	4		7	12	96	4	9	4	1	2			135
Alaska region	61		2	4	4	20	6	12	3	0			51
	65		1	2	21	2	37	13	12	8			96
	66												(*)
	67		0	0	4	2	3	25	7	9			50
	70		1	1	14	0	14	12	10	133			185
													900
						Factor scores, age 1.3							
B.C. region	9		129	46	12	4	1	0	0	1			193
	10		102	67	14	2	4	0	0	1			190
	4		4	15	94	6	5	2	1	8			135
Alaska region	61		2	0	3	32	4	7	3	0			51
	65		2	1	18	7	31	10	8	19			96
	66												(*)
	67		0	0	9	9	4	9	11	8			50
	70		0	0	14	1	13	4	5	148			185
													900

(*)Only one object was available so this area could not be tested in this age-group.

In both age-groups, four factors accounted for 97% of the variable and additional factors would have added only a negligible increment.[4] The fourth factor was retained because it added 10% in each case. After rotation, each fourth factor "accounts for" a variable. For age 1.2 fish, the sum of the squares of the factor loadings was 1.25 and was 0.68 for the fourth principal component. The factor loadings were as follows:

Age 1.2	Orthogonal Factor Matrix (Varimax)			
Variable	1	2	3	4
A_1	–0.1551	–0.0673	–0.9316	0.2336
A_2	–0.1214	0.9397	–0.0282	0.3116
A_3	0.9664	0.0083	0.1431	–0.1208
C_1	–0.1137	–0.0552	–0.9710	0.0964
C_2	0.1731	0.8184	0.2219	–0.4885
C_3	0.8646	–0.0209	0.1378	–0.3330
A_2/C_2	–0.3073	0.0783	–0.2509	0.9140

Age 1.3				
Variable	1	2	3	4
A_1	0.9291	–0.1506	0.1051	0.2522
A_2	–0.1255	–0.1769	–0.9697	0.0882
A_3	–0.1837	0.9472	0.0641	–0.0918
C_1	0.9511	–0.1582	0.1324	0.1337
C_2	–0.3222	0.1326	–0.6001	–0.7148
C_3	–0.1157	0.9121	0.1064	–0.2736
A_2/C_2	0.2315	–0.2993	–0.2042	0.8985

[4]The eigenvalues for the principal components were: age 1.2: 3.25, 1.64, 1.21, 0.68, 0.14, 0.08, 0.01; age 1.3: 3.35, 1.85, 0.83, 0.75, 0.13, 0.07, 0.02.

All of the factor analyses were similar in that four main factors resulted with key variables as follows: A_1, C_1; A_2, C_2; A_3, C_3; and A_2/C_2. Thus the complexity of the data is almost halved and the meaning of the factors is obvious in this straightforward case. There are four variables, not seven, and the pairs of values for each year's scale measurement are associated in the case of the first three factors.

The hypothesis of homogeneity of dispersions was rejected for the original variables in both age-groups and for the factor scores in the age 1.2 group. The factored data consistently showed less significant variation among groups than the original variables (see Table 1). On the other hand, the significance of the differences among the group means was greater for the factored data. Presumably, the reduction of the dispersion variability increased the separation among the group means. The range of values for the determinants of the dispersion matrices (D values) for each analysis is given in the table. The single year, 1964, shows considerable variability, whereas one might expect it to show much less than the 4-year data. Wilk's Generalized η is similar to a multiple correlation in univariate analysis of variance. Thus η^2 expresses the proportion of variance explained. It is higher for the functions based on seven original variable scores than for the four factor scores.

In the 4-years' data, the discriminant functions, using the seven original variables, correctly assigned 60% of the basic sample fish in the 1.2 age-group and 62% in age 1.3 to their true runs, while those using the four factor scores identified 50% and 57%, respectively. The results are shown in Table 2.

TABLE 4. Discriminant functions performance by region, 1961–64 test data, using original variables and factor scores.

True region	Classified: B.C. region		Alaska region		Total		Classified: B.C. region		Alaska region		Total	
	Original variables, age 1.2						*Original variables, age 1.3*					
B.C.	582	87.9%	80	12.1%	662	100%	481	92.9%	37	7.1%	518	100%
Alaska	79	16.7%	395	83.3%	474	100%	54	14.1%	328	85.9%	382	100%
					1136						900	
	Factor scores, age 1.2						*Factor scores, age 1.3*					
B.C.	582	87.9%	80	12.1%	662	100%	483	93.2%	35	6.8%	518	100%
Alaska	85	17.9%	372	81.4%	457	100%	49	12.8%	333	87.2%	382	100%
					1136						900	

TABLE 5. Comparison of classification of age 1.2 and 1.3 sockeye salmon by discriminant functions with true origin, 1964 basic data, using original variables (age 1.2, number correctly assigned = 121/157; age 1.3, number correctly assigned = 104/137) and factor scores (age 1.2, number correctly assigned = 97/157; age 1.3, number correctly assigned = 83/137).

		B.C. region				Alaska region				
		Area assigned								
	True area	29	9	10	4	64	65	66	70	Total
		Original variables, age 1.2								
B.C. region	29	10	1	1	3	2	2	1	20	20
	9	2	16	1	1	0	0	0	0	20
	10	1	3	14	2	0	0	0	0	20
	4	1	0	0	19	0	0	0	0	20
Alaska region	64	2	0	0	0	17	0	0	1	20
	65	0	0	0	2	2	12	0	4	20
	66	0	0	0	1	0	0	16	0	17
	70	0	0	0	2	0	1	0	17	20
										157
		Factor scores, age 1.2								
B.C. region	29	6	1	1	3	2	5	0	2	20
	9	2	15	2	1	0	0	0	0	20
	10	0	6	11	2	0	0	1	0	20
	4	3	0	2	13	0	1	0	1	20
Alaska region	64	3	0	0	0	15	1	0	1	20
	65	1	0	0	2	0	10	0	7	20
	66	0	0	0	0	0	2	12	3	17
	70	1	0	0	2	1	1	0	15	20
										157
		9	10	4	61	65	66	70	Total	
		Original variables, age 1.3								
B.C. region	9	17	3	0	0	0	0	0	20	
	10	2	12	3	1	1	0	1	20	
	4	0	2	17	0	1	0	0	20	
Alaska region	61	0	1	1	14	0	3	0	19	
	65	0	0	2	0	14	1	3	20	
	66	0	2	0	0	0	16	0	18	
	70	0	0	0	0	3	3	14	20	
										137
		Factor scores, age 1.3								
B.C. region	9	18	2	0	0	0	0	0	20	
	10	4	9	5	1	1	0	0	20	
	4	0	0	14	0	3	2	1	20	
Alaska region	61	1	1	1	14	1	1	0	19	
	65	0	0	4	0	8	4	4	20	
	66	0	2	1	5	0	9	1	18	
	70	0	0	0	1	6	2	11	20	
										137

TABLE 6. Comparison of classification of age 1.2 and 1.3 sockeye salmon by discriminant functions with true origin, 1964 test data, using original variables (age 1.2, number correctly assigned = 143/308, age 1.3, number correctly assigned = 106/227) and factor scores (age 1.2, number correctly assigned = 153/308; age 1.3, number correctly assigned = 109/227).

		B.C. region				Alaska region				
		Area assigned								
	True area	29	9	10	4	64	65	66	70	Total
		Original variables, age 1.2								
B.C. region	29	18	0	12	7	5	6	2	0	50
	9	5	15	6	0	0	0	0	1	27
	10	10	20	11	4	0	4	0	1	50
	4	5	0	4	15	0	4	0	2	30
Alaska region	64	6	0	0	2	31	4	0	7	50
	65	3	1	0	4	1	26	4	11	50
	66	0	0	0	0	0	0	1	0	1
	70	3	0	0	0	5	7	9	26	50
										308
		Factor scores, age 1.2								
B.C. region	29	10	5	12	7	5	8	1	2	50
	9	2	16	6	0	0	2	0	1	27
	10	6	17	17	7	0	2	0	1	50
	4	2	0	1	19	0	6	0	2	30
Alaska region	64	10	0	0	1	33	2	1	3	50
	65	3	0	1	2	1	22	3	18	50
	66	0	0	0	0	0	0	1	0	1
	70	2	0	0	0	3	2	8	35	50
										308
		9	10	4	61	65	66	70	Total	
		Original variables, age 1.3								
B.C. region	9	24	22	0	3	0	1	0	50	
	10	21	20	4	3	2	0	0	50	
	4	0	3	17	1	4	4	1	30	
Alaska region	61	0	0	1	0	0	0	0	1	
	65	0	3	9	4	11	6	13	46	
	66	0	0	0	0	0	0	0	0	
	70	0	0	2	2	5	7	34	50	
									227	
		Factor scores, age 1.3								
B.C. region	9	30	16	0	2	2	0	0	50	
	10	20	17	3	1	4	4	1	50	
	4	0	2	20	5	2	0	1	30	
Alaska region	61	0	0	1	0	0	0	0	1	
	65	0	6	11	3	9	12	5	46	
	66	0	0	0	0	0	0	0	0	
	70	0	1	4	0	6	6	33	50	
									227	

But in the test samples the relative performance of the methods was reversed; the original variable functions classifying 46% and 53% correctly in the two age-groups while the factor-score functions did 47% and 57% (see Table 3). Only one of four comparisons showed a significant difference between the original variables and the factor scores in the age 1.2 group of the basic data. As in the earlier study of the pinks, the identification by region was much more reliable than by area. Table 2 and 3 are divided into British Columbia and Alaska regions. Table 4 shows that the degree of misclassification of test fish by region varied from 7 to 18% and differed little between the two methods. Alaskan fish were more apt to be classified as British Columbian than the reverse.

Comparison of the accuracy of 1964 results, area by area (see Table 5 and 6) with all years is also of interest. The figures below summarize Table 2, 3, 5, and 6 in this respect:

All years	Percentage correctly classified by area	
A. Age 1.2	Original variables	Factor scores
Basic samples	59.96	50.46
Test samples	45.60	47.01
B. Age 1.3		
Basic samples	62.28	56.80
Test samples	52.67	56.89

1964 only	Percentage correctly classified by area	
A. Age 1.2	Original variables	Factor scores
Basic samples	77.07	61.78
Test samples	46.43	49.68
B. Age 1.3		
Basic samples	75.91	60.58
Test samples	46.70	48.02

We see that the original variable functions are consistently more accurate than the factor-score ones on the basic samples, the data for which the functions were derived. This difference is more marked in the 1964 data. In the test samples — the "unknowns" — the factor-score functions did better than the original variable ones. The performance of the factor-score functions in the basic samples thus held up much better in the test samples than the original variable ones.

Discussion

This study has substantive and methodological implications. The former were emphasized in the earlier paper (H. T. Bilton and H. B. Messinger, unpublished data). Essentially we show that sockeye salmon can be classified correctly by run about half the time with scale measurements while the accuracy by region (British Columbia vs. Alaska) is better than 80%.

The methodological result that we feel is significant is the remarkable consistency in accuracy of prediction for factor-score discriminant functions between the basic data from which they were derived and the test data. Looking at the percentage differences in the Table 4 figures, we obtain:

Basic vs. Test Sample

All years	Correct classification by area (% difference) Original variables	Factor scores
Age 1.2	14.36	3.45
Age 1.3	9.61	–0.09
1964 only		
Age 1.2	30.64	12.10
Age 1.3	29.21	12.56

That a difference can be zero in one case is perhaps due to the removal of outliers in the basic samples but not in the test samples. The important thing is the contrast in each row between the original variables and the factor scores. Since the factor scores are estimated by a procedure analogous to multiple regression, they represent a weighted average of the values of the original variables. This process presumably is smoothing out some of the effects of random error in individual measurements so that the "true" discriminant functions can be more accurately estimated. This strategy would seem to have wide applicability in discriminant analysis.

The complexity of the data is halved without significant loss of information. If anything there is a slight gain since the classification performance of the factor-score functions in the test data is a little better than the original-variable functions. Although the relative importance of the different factors in the discriminant functions was not a concern of this study, the statistical independence of the sets of factor scores would permit interpreting their relative importance by the size of the coefficients in the discriminant functions.

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Changes in Plasma Constituents During Spawning Migration of Pacific Salmons

E. TRIPLETT[1] AND J. R. CALAPRICE[2]

Department of the Environment
Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C.

TRIPLETT, E., AND J. R. CALAPRICE. 1974. Changes in plasma constituents during spawning migration of Pacific salmons. J. Fish. Res. Board Can. 31: 11–14.

Blood constituents were measured in salmon (*Oncorhynchus gorbuscha* and *O. nerka*) at various times during the spawning migration. Values for blood sodium, potassium, calcium, glucose, protein, and hematocrit suggested that during early stages of migration through post-spawning these fish experience a syndrome reminiscent of mammalian hypoadrenocorticism. The reverse was true of fish in advanced stages of senescence. Older fish also suffered ascorbate deficiency with a resultant necrosis of the skin. This deficiency was corrected by intraperitoneal injection of ascorbate. Various forms of hormone therapy applied to senescent fish were unsuccessful in prolonging their life appreciably.

TRIPLETT, E., AND J. R. CALAPRICE. 1974. Changes in plasma constituents during spawning migration of Pacific salmons. J. Fish. Res. Board Can. 31: 11–14.

Nous avons mesuré les paramètres sanguins de saumons (*Oncorhynchus gorbuscha* et *O. nerka*) à divers stades de leur migration de fraie. Les valeurs de sodium, potassium, calcium, glucose, protéines et hématocrite du sang suggèrent que, durant les premiers stades de la migration jusqu'aux stades qui suivent la fraie, ces poissons éprouvent un syndrome qui rappelle l'hypoadrénocorticisme des mammifères. L'inverse est vrai pour des poissons à un stade de sénescence avancé. Les poissons âgés souffrent de plus d'une déficience d'ascorbate, résultant en une nécrose de la peau. Cette carence peut être corrigée par injection intrapéritonéale d'ascorbate. Diverses formes de thérapie hormonale appliquées aux poissons sénescents n'ont pas réussi, à venir jusqu'ici, à prolonger leur vie de façon appréciable.

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MECHANISMS that control postspawning aging and death in Pacific salmon are not clearly understood, but several factors are contributory. Firstly, the products of the gonads must be involved since gonadectomy of gravid animals may result in their survival (McBride et al. 1969; Robertson 1961). Secondly, the ionic constituents of the environment must have an effect since the permanently landlocked form of sockeye salmon (kokanee) may spawn several times in successive years (Robertson 1957). Thirdly, histological and biochemical observations suggest that the pituitary–adrenal cortical axis might be involved in the aging process (McBride et al. 1969; Hane and Robertson 1959; Idler et al. 1962; La Roche et al. 1963; Robertson et al. 1961a, b; Robertson and Wexler 1962; Schmidt and Idler 1962; Fagerlund 1967; van Overbeeke and McBride 1967; McBride 1967). Finally, mature Pacific salmon do not eat during their spawning migration, and inanition is well known to aggravate the effects of adrenal cortical malfunction.

A way to test the hypothesis that aging and post-spawning death is a result of adrenal cortical malfunction aggravated by inanition, gonadial products, and unfavorable ionic environment is to measure blood constituents known to be controlled (in mammals) in part or completely by adrenal steroids. With this in mind we measured plasma sodium, potassium, calcium, glucose, protein, and hematocrit in two species of Pacific salmon at various stages of maturation. A small series of pilot experiments were also carried out to attempt to correct the physiological problems.

Materials and Methods

Pink salmon (*Oncorhynchus gorbuscha*) were obtained at Salmon River, Vancouver Island, B.C. as well as Babine Lake, B.C., and sockeye salmon (*O. nerka*) from Babine Lake. Nets and traps were used to capture both species.

[1]Present address: Department of Biological Sciences, University of California, Santa Barbara, Calif.

[2]Present address: Huntsman Marine Laboratory, St. Andrews, N.B.

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TABLE 1. Values for blood constituents of pink salmon during various stages of maturation and senescence.

Constituent	Stage I	Stage II	Stage III	Stage IV	Stage V
Sodium (mM)	128±25.1(40)[a]	145±13.6(49)	138±13.5(20)	143±10.5(19)	110±28.0(47)
Potassium (mM)	18±19.1(34)	14±15.0(37)	20±17.5(8)	19±15.1(9)	53±31.4(40)
Calcium (mM)	1.7±.56(34)	2.1±.92(29)	2.1±-(2)	2.0±.26(8)	1.1±.26(29)
Glucose (mg/ml)	.89±.46(37)	.74±.47(51)	.36±.19(16)	.39±.28(18)	.25±.26(29)
Protein (mg/ml)	32±13.0(51)	19±13.1(52)	18±11.5(18)	11±5.9(18)	44±36.5(47)
Hematocrit (%RBC)	36±5.8(37)	32±5.2(44)	26±7.4(18)	29±8.8(17)	54±14.4(46)

[a]Mean, one standard deviation of mean, and sample size.

Fish (Table 1) were classified as to stages of maturation and senescence as follows: Stage 1 — recently arrived in fresh water, nongravid; Stage 2 — gravid; Stage 3 — recently spawned; Stage 4 — spawned, showing external signs of degeneration but still actively swimming; Stage 5 — spawned, in advanced stages of external degeneration and too weak to maintain headway in moderate stream currents.

One milliliter of blood was taken from each fish by cardiac puncture, and the fish was then returned to the stream without having suffered any apparent ill effects. Bloods were transferred to heparinized tubes, and hematocrits were obtained within a few minutes after bleeding the fish. The remaining blood was centrifuged, and the plasma thus obtained was frozen immediately. Hemolyzed samples were always discarded. A total of 429 samples was obtained.

Sodium, potassium, and calcium concentrations in plasma were obtained with a Jarrel-ash flame spectrophotometer using the flame emission mode. Glucose and protein determinations were done according to Hawk et al. (1951) and Lowry et al. (1951), respectively. Hematocrits were performed with capillary tubes and an international centrifuge equipped with a hematocrit rotor.

Results

The concentrations of blood constituents as a function of maturation and senescence were essentially the same for the two species. Therefore, only the figures for pink salmon (Table 1) are reported here. Sodium values show the following trend. Stage 1 fish had a slightly lower Na^+ than would be expected for a "sea run" fish of this type. By Stage 2 the value was normal and remained so through Stage 4. At Stage 5 there was an abrupt decline in Na^+ with a large concomitant increase in standard deviation.

A similar trend is seen in plasma calcium, though the standard deviation did not change at the terminal stage. The reverse trend is seen for plasma potassium. K^+ in migratory fish is always somewhat higher than in oceanic salmonids, but at Stage 5 there was a 2.8-fold increase in average K^+ values with an accompanying increase in standard deviation.

Protein and hematocrit followed very similar time courses. During the migration there was a gradual decline in both values until Stage 5, when both increased remarkably. Hematocrits as high as 89% were observed in Stage 5 animals.

Values for glucose were among the most noteworthy in terms of their pathological implications. Glucose remained essentially normal or slightly elevated during the earlier stages of migration and at sexual maturation (Stage 2). But at Stage 3 there was an abrupt decline and at Stage 5, the values were very low. In fact, a few fish contained no plasma glucose that could be detected by the technique we employed.

With these results in mind a small series of pilot experiments were performed in an attempt to correct the physiological problems that these fish face after spawning. Pink and chum salmon (*O. keta*) were used in these experiments with similar

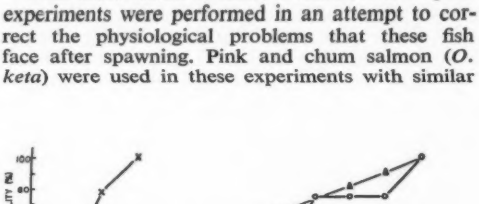

FIG. 1. Mortalities of pink salmon (*Oncorhynchus gorbuscha*) in water of various tonicities. ×, sea water (310 mM Na^+); △, fresh water (2.6 mM Na^+); ○, dilute sea water (140 mM Na^+).

results, only those for pink salmon being reported. All were as near Stage 4 as possible. Temperatures of the holding tanks ranged from 4 to 12 C.

The first experiments were carried out to determine whether ionic strength of the environment influenced survival of aging animals. Three concentrations were tested: sea water (310 mM Na^+), approximately isosmotic dilute sea water (140 mM Na^+), and fresh water (2.6 mM Na^+). Sample size in each category ranged from 9 to 11. As expected, survival was highest in the isosmotic medium (Fig. 1). The several inflection points on the curves correspond to times when tank maintenance was performed within the line of sight of the animals. They were extremely sensitive to stresses of this type (in contrast to immature fishes of the same species kept in the same tank).

A second experiment tested the survival effect of adrenal steroids and other substances injected intraperitoneally into fish (averaging 3.2 kg) maintained *in isomotic dilute sea water. Control animals* received 2000 units penicillin-G, 50 μg streptomycin, and 2000 U fungizone dissolved in 5 ml Earle's balanced salt solution (BSS). Experimental animals were treated with this same solution containing various test substances. Each fish (control and experimental) was force-fed (commercial fish paste) immediately prior to injection.

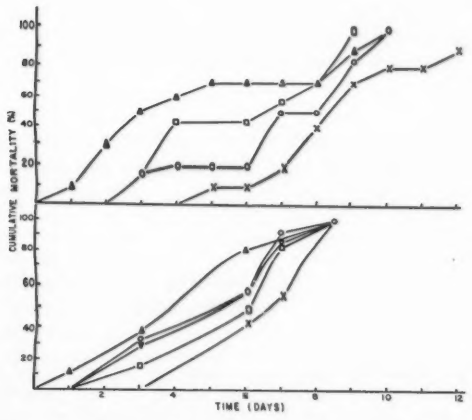

FIG. 2. Mortalities of pink salmon injected intraperitoneally with various compounds. *Upper:* ×, Cyclic adenosine monophosphate (2.5 mg); ○, cortisone (2.0 mg) and thyroglobulin (2.0 mg); □, cortisone (2.0 mg); △, control. *Lower:* ×, whole fresh pituitary glands from sea-run coho salmon (5); □, deoxycorticosterone (2.0 mg); ▽, thyroglobulin (2.0 mg) and deoxycorticosterone (2.0 mg), ○, thyroglobulin (2.0 mg); △, control.

Two of the substances tested (Fig. 2) had a statistically significant effect on survival for in each case the probability that all survivorship curves were drawn from the same population was less than 0.0005 (Friedman two-way analysis of variance). With the exceptions of cyclic AMP and whole fresh pituitary glands the substances tested had no appreciable effect on survival. These included deoxycorticosterone (2.0 mg/fish or 0.2 mg/fish), cortisone (2.0 mg/fish or 0.2 mg/fish), cyclic adenosine monophosphate (2.5 mg/fish), estrone (0.5 mg/fish), and thyroglobulin (2.0 mg/fish or 0.2 mg/fish). The whole fresh pituitary glands were taken from sea-run coho salmon (5 glands/fish).

Repeated injections at 3-day intervals were attempted, but the extreme sensitivity of senescent fishes to the stress of handling made these experiments impossible to interpret.

It was postulated that migrating salmon must have a large demand for ascorbate since this molecule *is importantly involved in hydroxylation mechanisms* in steroid synthesis and since, judging from morphological criteria, large amounts of collagen are synthesized during the spawning migration (ascorbate is a cofactor for the hydroxylation of proline, and collagen is rich in hydroxyproline residues). Accordingly a series of 10 Stage 4 fish were injected with 2.0 mg/fish ascorbate dissolved in BSS antibiotic solution. Although there was no change in survival as compared to the controls, the ascorbate treated series failed to experience the discoloration and sloughing of the skin that characterizes normally aging salmon.

Conclusions

These results suggest that postspawning Pacific salmon experience a serious malfunction of mechanisms that control the levels of certain blood constituents. Attempts to prolong the life of such salmon by the administration of hormones and other reagents were not entirely successful.

Administration of ascorbic acid to senescent salmon, while it did not prolong their lives, did prevent the extensive necrosis of the skin that is characteristic of this species.

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Myxosoma cerebralis: Isolation and Concentration from Fish Skeletal Elements — Sequential Enzymatic Digestions and Purification by Differential Centrifugation

MARIA E. MARKIW AND KEN WOLF

Bureau of Sport Fisheries and Wildlife
Eastern Fish Disease Laboratory, Leetown, Kearneysville, W. Va. 25430, USA

MARKIW, M. E., AND K. WOLF. 1974. *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements — sequential enzymatic digestions and purification by differential centrifugation. J. Fish. Res. Board Can. 31: 15–20.

An effective sequential procedure for recovery of *Myxosoma cerebralis* spores from infected trout was developed, and quantification of spores was carried out at each step of release and concentration. Methods are described for fresh and frozen material. Effective concentration of from 1100- to 9000-fold and an estimated efficiency of recovery of about 80% has been achieved. Tabular and graphic data are presented with recommendations for diagnostic applications. The immediate applications of these procedures are in implementing more effective detection and in preparing antigen for the immunologic studies that should provide the most sensitive detection.

MARKIW, M. E., AND K. WOLF. 1974. *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements — sequential enzymatic digestions and purification by differential centrifugation. J. Fish. Res. Board Can. 31: 15–20.

Nous avons mis au point une technique séquentielle pour la récupération des spores de *Myxosoma cerebralis* chez des truites infectées, et en avons effectué la quantification à chaque palier de libération et de concentration. Nous décrivons des méthodes applicables à du matériel frais et congelé. Nous avons réussi une concentration effective de 1100–9000 fois plus grande et une efficacité de récupération d'environ 80%. Nous présentons des données sous forme de tableaux et de graphiques, avec recommandations en vue d'une application diagnostique. Les applications immédiates de ces techniques sont, d'une part, une détection plus efficace et, d'autre part, la préparation d'un antigène pour des études immunologiques, qui donnerait la détection la plus sensible.

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Myxosoma cerebralis is a myxosporidian parasite of European origin that has been introduced into North America, and is now established in the effluent waters in several hatcheries and presumably in waters stocked with hatchery-infected fishes. Depending upon the severity of infection and upon salmonid host species — notably in rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*), *M. cerebralis* can produce mortality, cripple, or disfigure. The clinical disease is known as "black tail" or more commonly as "whirling disease," and is readily diagnosed on the basis of signs and behavior changes. Identification is based upon biophysical characteristics of the spores.

At present, histologic examination or microscopy for spores are the only techniques available for identification of *M. cerebralis* and hence for verification of whirling disease. Both approaches are time-consuming and have serious limitations in sensitivity of detection; the carrier state, pre-spore incubation stages, and light infections are all apt to be missed. As has been shown with many vertebrate pathogens, serologic procedures often provide a simple, rapid, and sensitive approach to detection and the most accurate identification. Methods that release and concentrate *M. cerebralis* spores with minimal residues of fish tissue or debris will greatly increase sensitivity of detection and also provide a means of obtaining antigen needed for developing serologic procedures. With that goal in mind, Landolt (1973) has developed a procedure whereby trypsin is used to release spores from decalcified skeletal elements and further biophysical steps are employed to minimize fish-tissue contamination and to concentrate spores. The methodology requires 18–24 h and effects upon spore antigenicity are as yet undertemined.

The purpose of our report is to describe an alternative and more rapid procedure for releasing and concentrating spores of *M. cerebralis*. The

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newer method is suitable either for young fish with predominantly cartilaginous skeletons or for older fish in which ossification has occurred.

Materials and Methods

Fish

Fresh and frozen infected fingerling rainbow trout were used for all work. Fresh, 4- and 5-month-old specimens were experimentally infected at 12.5 C by the method of Hoffman and Putz (1971). Frozen 4-month-old fingerlings with natural infection were also used. They were provided by the Pennsylvania Fish Commission, Benner Spring Fish Research Station, Bellefonte, Pa.

Removal of Soft Tissue and Mincing Cartilage

Fish were immersed in water of 45–50 C for 5–10 min (MacLean 1971; Taylor personal communication). Following heating, eyes, skeletal muscle, and adherent soft tissues were teased away. Gill arches were excised intact and pooled with the cleaned skeletal parts. Skeletal parts were cut into 3–5 mm pieces with scissors.

Peptic Digestion

Minced skeletal fragments were initially digested in freshly prepared pepsin (Pepsin, N.F.) 0.5% (w/v) at *p*H 1.8–1.85 in 0.5% HCl solution. One volume of 25–30 ml pepsin solution was used for each gram of skeletal fragments; digestion was carried out in 250-ml Erlenmeyer flasks containing three marbles, in a 37.5 C shaker bath for 1–3 h. When young fish were used, 1 h was adequate. Skeletal fragments from older fish required two to three times greater digestion time. If cartilage remained after 2 h, fluid was harvested and an equal volume of fresh pepsin added. Digestions tried at 23 C required 4–4.5 h. At the end of pepsin digestion, all material was centrifuged in a horizontal head at 22 C at 1200 *g* for 10 min. Supernatant fluid was aspirated and each volume of pelleted sediment was resuspended in 12.5–15 ml water for secondary digestion.

Tryptic Digestion

Secondary digestion was effected with purified trypsin 0.5% (w/v) dissolved in calcium- and magnesium-free Tyrode's solution as modified by Rinaldini (1959).[1] Trypsin solution was filtered through Whatman #114 filter paper. Phenol red at a concentration of 0.002% was added as a *p*H indicator.

Resuspended peptic sediments were mixed with equal volumes of trypsin solution to give a final concentration of 0.25%. The starting *p*H was adjusted to 8.0–8.5 with 1N NaOH. Digestion was at 22 C for 30 min with occasional gentle agitation.

[1]Composition: NaCl 8.0 g, KCl 0.2 g, $C_6H_5Na_3O_7$-$2H_2O$ (sodium citrate) 1.0 g, $NaH_2PO_4H_2O$ 0.05 $NaHCO_3$ 1.0 g, glucose 1.0 g, water to make 1000 ml.

Secondary digestion with 0.25% trypsin of material from fish that had been frozen resulted in excessive spore damage; during counting procedures spores were seen to disintegrate. A 0.05% concentration was tested and found to be satisfactory for use on material from frozen fish.

Tryptic digestion was arrested by addition of fetal bovine serum at a final concentration of 20% (v/v) or 1% (w/v) bovine serum albumin powder. Tryptic digests were filtered through a gauze-thin layer of glasswool, and centrifuged at 1200 *g* for 10 min at 22 C. Pelleted material was resuspended in 20–25 vol of half-strength Hanks' balanced salt solution containing 20% fetal bovine serum.

Purification, Isolation, and Concentration by Differential Centrifugation

Five ml of resuspended tryptic digest pellet were layered onto 3 ml of 55% (w/v) aqueous dextrose solution contained in a 12 ml conical centrifuge tube or for quantification, onto 1 ml dextrose in a 6.5-ml Shevky-Stafford albumin tube. Both were centrifuged at 1200 *g* for 30 min at 22 C. Five-gram aliquots of cartilage from healthy fish were finely minced, transferred to Erlenmeyer flasks, and seeded with 1000, 10,000, or 100,000 spores. Control cartilage was not seeded with spores. A parallel series of flasks without cartilage received the same numbers of spores. The contents of both sets of flasks were treated first with pepsin and then with trypsin. Residues were centrifuged through dextrose solution and aliquots were quantified for estimates of remaining spores.

Quantification

Spore number was determined from duplicate or quadruplicate counts of 0.4 mm^3 in a Neubauer hemacytometer. Only typical spores were counted: those that had uniform size and two clearly recognizable polar capsules of normal size. Spores were occasionally encountered with three or four polar capsules or with abnormally small capsules.

Efficiency of Recovery

There is no known procedure for establishing uniform infection with *M. cerebralis* or for determining beforehand the actual number of spores occurring in an infected fish. Accordingly, the only practical method of assessing the efficiency of recovery of our procedures was to seed uninfected cartilage fragments with known numbers of spores. To assure minimal degradation, spores for such seeding were obtained by mechanical means; they were gently rubbed from infected gill arches with a porcelain pestle. Debris was allowed to settle, and the suspended spores were decanted and counted.

Effects of Freezing and Storage on Spore Recovery from Cartilage

Cartilage from 7- to 8-month-old trout was pooled, finely minced, distributed in 2-g aliquots in screw-cap vials, and frozen at –2 C. A control aliquot was not

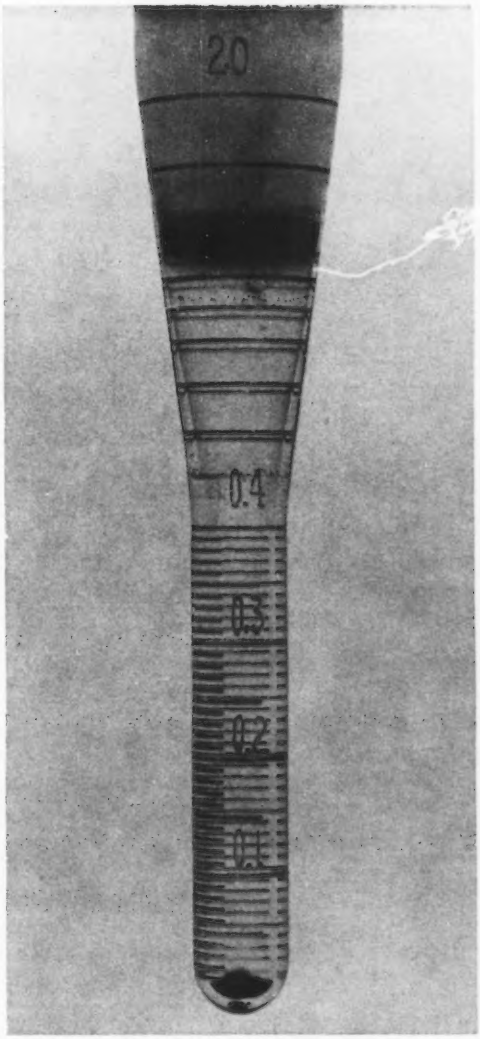

FIG. 1. Typical results of purification of *Myxosoma cerebralis* spores by centrifugation of tryptic digest residues layered onto 55% dextrose column. Spores pelleted at bottom of tube have passed through optically clear sugar solution, but dense band of debris and opaque material has remained at the top.

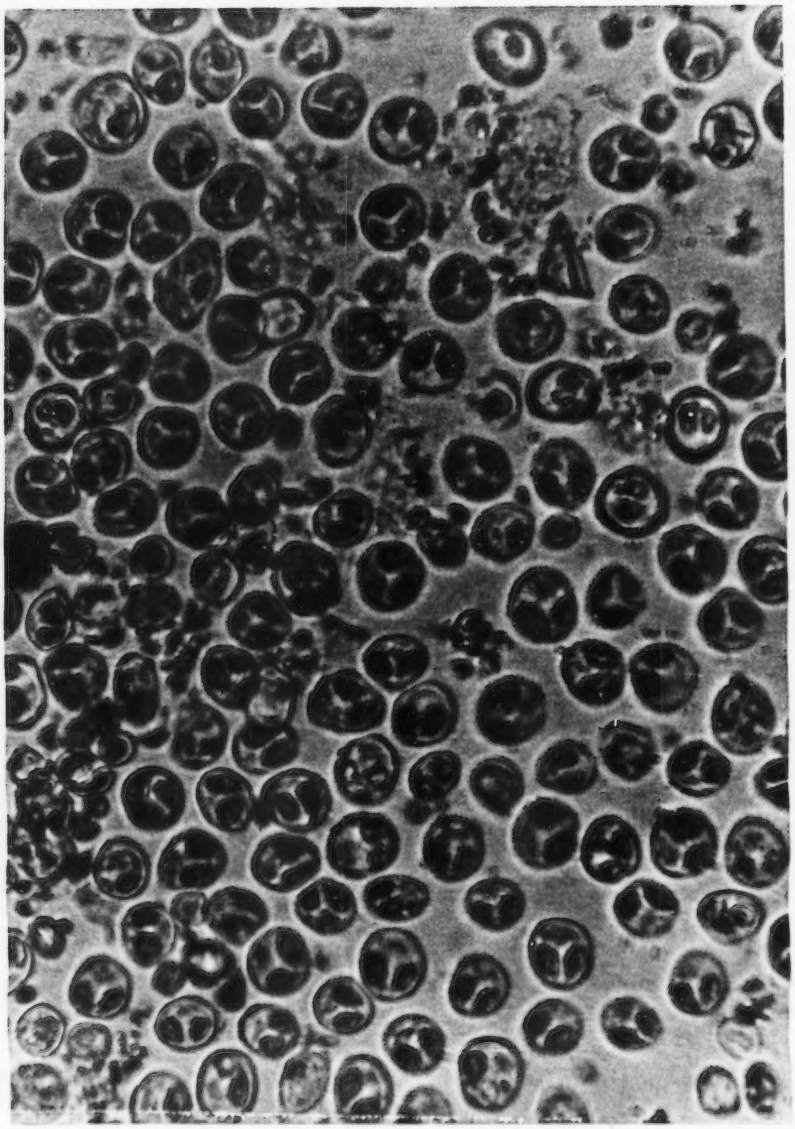

FIG. 2. Concentrated suspension of *M. cerebralis* spores obtained by sequential digestions and purification by centrifugation through 55% dextrose.

frozen but was processed immediately. Periodically, frozen samples were thawed and processed.

Results

Sequential enzymatic digestion of minced skeletal elements released spores of *M. cerebralis*, which were concentrated in a relatively pure condition by differential centrifugation through dextrose solution (Fig. 1 and 2). The total time required was about 8 h and the recovery was at least 80% efficient.

Removal of soft tissue from skeletal elements gave an 8.3–8.9-fold reduction of bulk and effected an equivalent spore concentration; the resulting cartilaginous-bony elements constituted about 12% of the total fish weight. Removal of soft tissue did, however, result in loss of some spores.

The incidence of spores in tissues in 10 4–5-month-old fingerlings was determined to provide guidelines for recovery and concentration. As determined by peptic digestion alone, excised gills contained from a trace to 96% of total spores (average 37%), the head itself, minus gills, contained 0–100% (average 27%), the axial skeleton minus the head contained 0–48% (average 16%), and soft tissues plus fins contained from 0–57% (average 20%). Most of the spores (an average of 79%) occurred in the axial skeletal elements (Table 1). That fact and the fact that digest residues from soft tissues made microscopic examination difficult lead to a decision to discard soft tissues from the balance of the study. However, soft-tissue removal was thereafter done gently to minimize spore loss from the surface of the head and axial skeleton.

Peptic digestion of cartilage uniformly resulted in a 9–11-fold additional spore concentration (Table 2). Cartilage from young fish (2–3-month-old) was digested in 1 h, and that from 3- to 4-

TABLE 1. Occurrence of *Myxosoma cerebralis* spores in various rainbow trout (*Salmo gairdneri*) tissues.

Fish Number	1	2	3	4	5	6	7	8	9	10	Average
Tissue											
Gills	19%	18%	42%	42%	26%	20%	59%	45%	Trace	96%	37%
Head	10%	39%	31%	10%	17%	47%	8%	11%	100%	None	27%
Spinal column	48%	34%	13%	None	None	33%	15%	17%	Trace	4%	16%
Soft tissues	23%	9%	17%	48%	57%	None	18%	27%	None	None	20%
Percentage in skeletal elements	77%	81%	83%	52%	43%	100%	82%	73%	100%	100%	$\bar{m}$ 79%
Number of spores per fish $\times 10^5$	20.3	12.4	13.5	2.3	2.9	2.8	4.5	1.5	0.2	0.9	

[a]Spores detected after centrifugation through dextrose. Pellet resuspended in 0.1 ml and an 0.8-μl aliquot counted. Number $\geq$ two spores per aliquot.

TABLE 2. Comparative yield of *M. cerebralis* spores during release and concentration sequence.

Material	Series	Weight or volume I[a]	Weight or volume II[b]	Spores per ml I	Spores per ml II	Concentration factor I	Concentration factor II
Intact fish		57.60 g	339.00 g	Unknown	Unknown	None	None
Minced skeletal parts		6.70 g	40.40 g	4.4×10^4	6.5×10^4	8.6×	8.3×
Peptic digest residue		0.60 ml	4.5 ml	6.1×10^6	5.8×10^6	11.2×	9.0×
Tryptic digest residue		0.22 ml	2.3 ml	3.2×10^7	2.0×10^7	2.7×	2.0×
Pellet after differential centrifugation through dextrose		0.5 ml	0.04 ml	1.1×10^8	1.2×10^8	4.4×	61.3×
					Cumulative concentration	1144×	9041×

[a]Series I data are mean values from three trials each with five fish.
[b]Series II data are mean values from one trial with 25 fish.

month-old fish required about 2 h. Seven- to 8-month-old fish required the longest time; pepsin solution was changed after 2 h and an additional charge of fresh pepsin was allowed to act for 30–60 min to effect complete digestion — a total time of 2.5–3 h.

Tryptic digestion resulted in release of additional spores; this was particularly true when older fish were used. This step gave about a two- to three-fold greater concentration than was present in peptic digest pellets. As shown in Fig. 3, trypsin concentration and time both affected spore recovery.

Centrifugation through dextrose, the final step in the recovery sequence, varied widely in degree of concentration. At a minimum, there was a four-fold concentration, at a maximum, 60-fold.

Quantification of the entire process showed an effective spore concentration per unit volume of from 1100 to 9000 times that present in the intact fish. This does not take into account the spores known to be present in soft tissues that were discarded during recovery of cartilage.

The efficiency of recovery from normal cartilage seeded with 2000 or 20,000 spores per ml and processed with pepsin, trypsin, and dextrose centrifugation was 81.5–86.75% (Table 3). A series of 32 counts gave a mean recovery of 83% with a standard deviation of ±1.12%. When the initial spore level was only 200 per ml, a few spores were always recovered, but the percentage was too low to permit valid calculation of the efficiency of recovery.

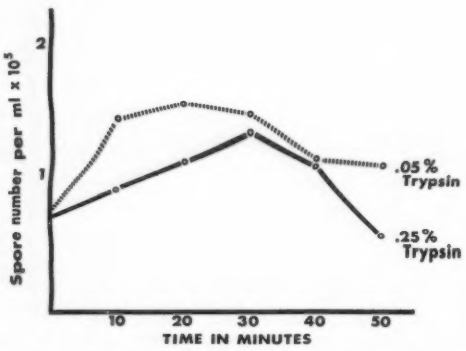

FIG. 3. Effects of trypsin concentration and time on release of spores from resuspended peptic digest pellets.

TABLE 3. Results of recovery efficiency tests using normal cartilage seeded with *M. cerebralis* spores.[a]

Number of spores seeded	Mean number recovered	Mean percentage recovery
Replicate 5-g aliquots of minced normal cartilage in 125 ml pepsin solution		
1,000	<2 per .4 mm³[b]	<0.2%
10,000	8,175	81.75%
100,000	82,250	82.25%
Replicate 125 ml aliquots of pepsin solution without cartilage		
1,000	<2 per .4 mm³	<0.2%
10,000	8,150	81.5%
100,000	86,750	86.75%

[a]Mean values for two trials. Dextrose pellet was resuspended in 0.5 ml for counting.

[b]At most, two spores were found in any 4-mm² field. Accordingly, dilution factor calculations would result in spore estimates that exceeded the number actually seeded.

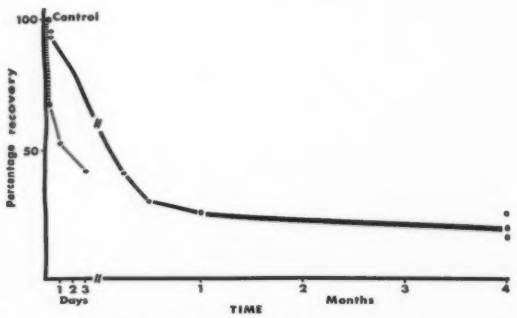

FIG. 4. Effect of freezing and storage on spore recovery by centrifugation through dextrose.

When frozen specimens were used as a source of spores, centrifugation in 55% dextrose solution was found to distort the normal shape of the sporoplasm and many damaged spores remained near the top of the liquid. Accordingly, the effect of freezing and thawing as it related to damage in dextrose solution was investigated.

Freezing at –2 C and thawing within 1 h reduced the recovery by one-third. Additional loss occurred during longer storage (Fig. 4). By 72 h, 60% of the spores were damaged beyond recovery. Still longer storage resulted in even greater damage, and after 4 mo the mean of three samples showed that only 18% of the spores could be recovered intact, with apparently normal morphology.

Discussion

The procedures reported here provide a highly efficient means of releasing and concentrating *M. cerebralis* spores in a relatively pure condition.

The significance of the work is at least twofold. It provides a means of preparing spore antigen that can be tested for applicability in immunological–serological studies. Secondly, the methodology greatly increases the probability of detection when spores are present in small numbers. This quantified sensitivity has no parallel in published reports on *M. cerebralis.*

In its entirety, the sequence of spore release and concentration requires up to 8 h, but that is still only 40–80% of the time reported by Rydlo (1971). Moreover, for diagnostic and detection work the entire sequence need not be carried out; instead, spores can be sought at each step. The methods have further value in being applicable, with suitable precautions, to frozen specimens.

The finding of microsporidian parasites in certain of the specimens is evidence that the procedures will be applicable to other small parasites.

Recommendation for Detection, and Diagnostic Applications

Based upon the present need for reliable means of detecting *M. cerebralis* spores, the following procedures are recommended:

1. Gill arches should be removed, grossly trimmed of their filaments, and the cartilage itself gently crushed in a mortar and pestle. The crushed tissue should be mixed with 5 vol of water, the gross fragments allowed to settle for 1 or 2 min, a sample taken from the top of the supernatant fluid and examined for spores. If spores are not quickly found, specimens should be heated and the skull and axial skeleton processed. About 1 min is required to strip soft tissue from a heat-treated fingerling.

2. Gill arches and skeletal elements should be minced and digested with pepsin, and the digest fluid examined for spores. From 1 to 3 h are required for pepsin digestion, depending upon fish age. If no spore is found, the total volume should be centrifuged, the pellet resuspended in about 10 vol of water and also examined. Spores will usually be found in this step, even in very lightly infected fish, but if not, digestion with trypsin should be carried out.

3. The peptic digest residue should be trypsinized for about 30 min, then serum added to a final concentration of 20% or powdered bovine serum albumin to a final concentration of 1% to stop tryptic action. At this time, the maximum number of spores will have been released, and the fluid should be examined microscopically.

4. In cases where very small numbers of spores are present, they will be difficult to find in the relatively diluted trypsin solution, and debris will interfere with microscopic search. The neutralized tryptic digest should be centrifuged, the pellet resuspended in about 20 vol of half-strength physiological salt solution containing either 20% serum or 1% bovine serum albumin. The resuspended pellet should be carefully layered onto 55% dextrose solution in 12 ml conical centrifuge tubes at the rate of 5 ml per 3 ml of solution. After centrifugation, the spores will be pelleted and easily found in microscopic examination.

Where spore detection in frozen specimens is critical, trypsin should be employed only at a 0.05% concentration, and centrifugation through dextrose should not be used because it will result in excessive spore damage and loss.

For diagnostic purposes, the material that has been pelleted through dextrose solution should be examined promptly. If spores are to be kept for other purposes,they should be resuspended in half-strength physiological salt solution with 20% serum or 1% bovine serum albumin.

Acknowledgments

We are indebted to Dr G. L. Hoffman for providing the infected specimens used in this work, especially for providing a wealth of information on *M. cerebralis* and on whirling disease. Release of one of us (M.M.) from other duties to carry out this work was sincerely appreciated.

Mr J. E. Sanders and Drs J. L. Fryer and R. L. Herman read the manuscript and offered valuable criticisms. Their help is greatly appreciated.

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Availability, Size Composition, Age Composition, and Growth Characteristics of Pacific Ocean Perch (*Sebastes alutus*) off the Northern Washington Coast During 1967–72

DONALD R. GUNDERSON

Washington State Department of Fisheries
Fisheries Center, University of Washington, Seattle, Wash. 98105, USA

GUNDERSON, D. R. 1974. Availability, size composition, age composition, and growth characteristics of Pacific Ocean perch (*Sebastes alutus*) off the northern Washington coast during 1967–72. J. Fish. Res. Board Can. 31: 21–34.

Cruises were conducted off the northern Washington coast each year during 1967–72 to study trends in the availability and biological characteristics of Pacific Ocean perch (*Sebastes alutus*).

There were marked differences in catch rate between cruises, and corresponding differences in age composition and age–length structure indicated that they resulted from differential availability of an old, slow-growing segment of the population to on-bottom trawling. Females from this segment of the population were particularly available when the cruise coincided with the period of peak spawning, and both sexes were available during the 1972 summer cruise.

Within cruises, adults from different portions of the bathymetric range showed similar age composition, but there was an inverse relationship between depth and size attained by age 15. Size compositions consequently contained a smaller proportion of large fish as depth increased.

Juveniles were confined to the shallow portions of the bathymetric range. There were obvious between-year changes in the length and age composition of juveniles, caused by the temporal progression of a dominant 1961 year-class.

Fluctuations in availability make it difficult to monitor changes in mortality and since age composition also varies with availability, the problem is compounded further. Unless all segments of the population are located and sampled in proportion to their abundance, estimates of mortality can be made only between those cruises where the old, slow-growing component of the population is equally available.

GUNDERSON, D. R. 1974. Availability, size composition, age composition, and growth characteristics of Pacific Ocean perch (*Sebastes alutus*) off the northern Washington coast during 1967–72. J. Fish. Res. Board Can. 31: 21–34.

De 1967 à 1972, on a effectué des croisières au large de la côte septentrional de l'Etat de Washington, en vue d'étudier les tendances d'accessibilité et les caractéristiques biologiques de la perche de mer du Pacifique (*Sebastes alutus*).

Le taux de capture accuse des différences notables d'une croisière à l'autre. Des différences correspondantes dans la proportion des âges et dans la structure âge-longueur indiquent une accessibilité différentielle au chalutage sur le fond d'un segment âgé, à croissance lente, de la population. Les femelles faisant partie de ce segment de la population sont particulièrement accessibles lorsque la croisière coïncide avec la période de pointe de la fraie. Les deux sexes ont été accessibles au cours de la croisière d'été de 1972.

Au cours d'une même croisière, les adultes provenant de profondeurs différentes ont la même composition en âge, mais il existe une relation inverse entre la profondeur et la taille atteinte à l'âge de 15 ans. La composition en taille comprend donc une plus faible proportion de grands poissons à mesure que la profondeur augmente.

Les jeunes sont confinés dans la portion peu profonde de l'étage bathymétrique. Il existe des changements notables, d'une année à l'autre, dans la composition en âge et en taille, changements attribuables à la progression dans le temps de la classe d'âge dominante de 1961.

Les fluctuations de l'accessibilité font qu'il est difficile de suivre de façon continue les variations de mortalité. Le problème se complique du fait que la composition en âge varie également avec l'accessibilité. A moins qu'on ne localise tous les segments de la population

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et qu'on en fasse l'échantillonnage dans la même proportion que leur abondance, on ne peut estimer la mortalité qu'entre les croisières où le segment âgé et à croissance lente de la population est accessible au même degré.

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IN 1967 the Washington Department of Fisheries significantly expanded its program of Pacific Ocean perch (*Sebastes alutus*) stock assessment, largely in response to increased foreign fishing activity off Washington and Oregon. As part of this program, a series of biological cruises was initiated.

The main objectives of these cruises were to monitor changes in abundance, size composition, and age composition at two sampling stations off the northern Washington coast (Fig. 1), and to examine variations in these parameters between sampling stations, depths, and seasons. Westrheim (1970), had conducted broad surveys of rockfish (Scorpaenidae) distribution and abundance from Cape Blanco, Oregon to Unalaska Island, Alaska during 1963–66. Detailed surveys similar to those undertaken in this study had been initiated in Queen Charlotte Sound, B.C. and off La Perouse Bank (Fig. 1).

Methods

GENERAL DESIGN

Cruises were conducted each year from 1967–72. The M/V *Commando* was used for all cruises except 1972–1 when the M/V *Westness* was used. The timing and extent of the cruises were as follows:

Cruise no.	Date covered	Drags made (in parentheses) at each sampling depth (fath)
67–1	May 31–June 10, 1967	100(3); 150(4) 200(1)
68–1	Feb. 25–Mar. 10, 1968	100(7); 150(6) 200(7); 250(7)
68–2	July 17–July 28, 1968	80(8); 120(8) 160(8); 200(8)
69–1	Mar 1–Mar. 12, 1969.	100(5); 150(6) 200(5); 250(5)
69–2	July 1–July 10, 1969	120(9); 160(10) 200(9)
70–1	Feb. 14–Feb. 27, 1970	150(8); 200(8) 250(7)
70–2	Sept. 27–Oct. 4, 1970	120(8); 160(8) 200(8)
71–1	Feb. 16–Feb. 22, 1971	150(4); 200(7) 250(6)
72–1	July 12–July 16, 1972	120(7); 160(8) 200(4)

The 1967 cruise served to refine the gear used and the sampling scheme employed. A 400 mesh eastern otter trawl with 3.5-inch mesh throughout and a 1.5-inch

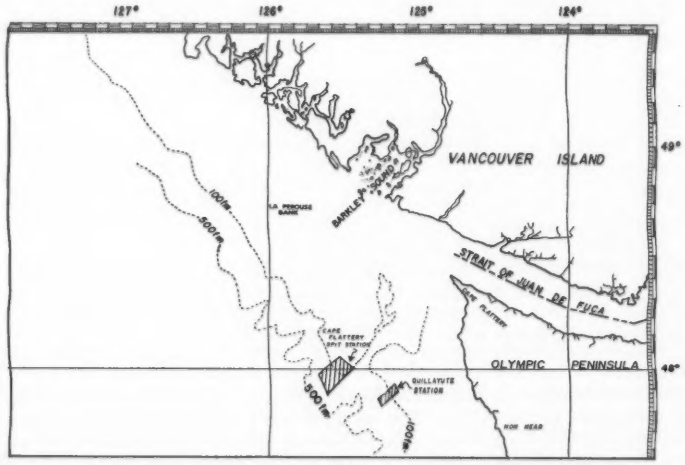

FIG. 1. Map of Washington and Vancouver Island coasts showing the location of sampling stations used in the study.

codend liner was used as a sampling instrument. Roller gear was attached to the footrope in the manner shown in Gunderson (1969a).

During cruises 68–1, 68–2, and 69–1, a sampling design with two stations and four depths within each station was employed. Four drags were made at each of these station-depth strata, and an attempt was made to make each during a different part of the day (morning, mid-morning, afternoon, or evening). All hauls were made during daylight hours, since off-bottom migrations of *S. alutus* have frequently been observed during the night (Lyubimova 1965; Taylor 1967). Drags were 30 min long during cruise 68–1 and 45 min long on all other cruises. Comparisons of distance covered per 45-min tow showed that the average distance traveled by the *Westness* was 21% greater than that covered by the *Commando*, and all effort data for the former vessel were adjusted upward by this amount.

The depths fished were chosen so as to bracket the bathymetric range occupied by *S. alutus*, and differed between seasons. During the summer cruises (68–2), the 80, 120, 160, and 200-fath (146, 219, 293, 366 m) depths were chosen. Pacific Ocean perch move into deeper water during the spawning period (Gunderson 1971b) so that the depths chosen for winter cruises (68–1 and 69–1) were 100, 150, 200, and 250 fath (183, 274, 366, 457 m). It was difficult to hold the boat over the target depth with precision, owing to the steep bathymetry in the study area, but deviation from this depth was no more than 15 fath for most drags.

The shallowest depth was not sampled during cruises 69–2, 70–1, 70–2, 71–1, and 72–1, since extremely small quantities of Pacific Ocean perch were caught at these depths during 1968 and 1969, and there was a high probability of damaging the gear there. This reduced the number of drags required to fulfill the design from 32 to 24. It was not always possible to complete all sampling in the time allotted for a cruise, owing to gear problems and inclement weather. All departures from the sampling scheme planned for a given cruise are detailed in the Groundfish Data Report (Gunderson 1969a, 1969b, 1971a, 1973) pertaining to that cruise.

Biological Sampling

For each effective drag, weight caught was estimated by species. The *S. alutus* catch was sorted into standard metal tubs, and the total number of tubs counted. Total pounds of Pacific Ocean perch caught were then estimated by using mean tub weight data from Westrheim et al. (1968).

Length and sex data were taken for each catch of *S. alutus*. When catches were smaller than six full tubs, the entire catch was examined; but with larger catches, Westrheim's (1967) technique of sampling the first two, middle two, and last two tubs filled from the catch (2–2–2 subsample) was followed. All fish were measured to the nearest centimeter (FL).

During cruise 72–1, otolith samples were collected randomly from each catch, and age composition was estimated directly for each drag. During all 1968–71 cruises, however, age–length keys were used to estimate age composition from length data. Separate keys were always constructed for each cruise–depth–sex stratum and whenever possible, these strata were further subdivided by sampling station. The number of otoliths collected during the 1968 cruises didn't allow the latter subdivision but for all 1969–71 cruises, separate age–length keys were employed for almost every cruise–depth–sex-sampling station stratum. The single exception to this was cruise 70–1 when a joint Quillayute–Spit key was used to estimate the Quillayute age composition at 250 fath.

Table 1 shows the number of length–sex observations and otoliths collected by sampling station

TABLE 1. Number of fish sampled for length, sex, and otoliths by cruise, depth, and sampling station.

		Quillayute		Cape Flattery Spit	
Cruise	Depth (fath)	Length–sex	Otoliths	Length–sex	Otoliths
68–1	150	316	182	85	85
	200	978	219	268	268
	250	108	108	282	145
	Total	1402	509	635	498
68–2	120	848	281	468	205
	160	609	180	259	212
	200	652	101	148	140
	Total	2109	562	875	557
69–1	150	531	437	60	60
	200	687	401	21	21
	250	237	136	9	9
	Total	1455	974	90	90
69–2	120	385	384	207	200
	160	1005	777	1011	741
	200	688	548	1149	710
	Total	2078	1709	2367	1651
70–1	150	161	142	155	155
	200	1146	481	833	465
	250	329	195	405	303
	Total	1636	818	1393	923
70–2	120	736	505	927	615
	160	779	518	982	626
	200	406	388	183	176
	Total	1921	1411	2092	1417
71–1	150	450	227	12	12
	200	1030	510	81	81
	250	191	189	371	152
	Total	1671	926	464	245
72–1	120	1348	448	908	299
	160	944	304	1279	301
	200	657	216	123	123
	Total	2949	968	2310	723

TABLE 2. Numbers caught per hour (sexes combined) by station, depth, and cruise.

Depth	Quillayute	Spit	Stations combined	Quillayute	Spit	Stations combined	Quillayute	Spit	Stations combined	Quillayute	Spit	Stations combined
Summer cruises		*68–2*			*69–2*			*70–2*			*72–1*	
80 fath	7	2	4	–	–	–	–	–	–	–	–	–
120 fath	403	348	376	128	91	110	309	445	377	964	810	887
160 fath	202	97	150	268	449	358	259	361	310	566	733	650
200 fath	217	49	133	246	401	324	135	60	98	420	135	278
All depths[a]	274	164	220	214	313	263	234	289	262	650	559	605
Winter cruises		*68–1*			*69–1*			*70–1*			*71–1*	
100 fath	19	2	10	17	0	8	–	–	–	–	–	–
150 fath	158	85	122	545	43	294	63	50	56	200	16	108
200 fath	1,328	179	754	229	28	128	887	292	590	407	37	222
250 fath	85	507	296	79	12	46	146	179	162	84	257	170
All depths[a]	523	257	390	284	28	156	365	174	270	230	103	167

[a]Data from 80 fath (summer cruises) and 100 fath (winter cruises) not included.

and depth for each cruise. A limited number of maturity observations were also made following the classification system described in Gunderson (1971b).

Catch Rates

Differences Between Depths

Catch rates for the two sampling stations combined were relatively high at 120, 160, and 200 fath during summer cruises and at 150, 200, and 250 fath during the winter (Table 2). Within these broad bathymetric ranges, there were large between-cruise variations in the availability of Pacific Ocean perch at a given depth, as shown by the data for both summer and winter cruise series.

Differences Between Cruises

Extreme variation in catch rate occurred between cruises, with average catch rates at the three major fishing depths ranging from 156 fish per hour during cruise 69–1 to 605 fish per hour during cruise 72–1 (Table 2). Cruise 72–1 was quite exceptional since catch rates were much higher than during any other summer cruise (Table 2 and 3). Westrheim et al. (1972) reported a similar phenomenon during 1972, encountering extremely high catch rates of *S. alutus* during a September cruise off southwest Vancouver Island (30–60 miles north of the study area).

Variations in catch rate were more common between winter cruises, largely because of variations in the availability of adult females (Table 3). Comparison of maturity data (Table 4) with catch data from the corresponding winter cruises indicates that female *S. alutus* are highly available for a short time when embryos are being released, and that catches were particularly high when the cruise dates coincided with the period of embryo release (spawning). Mature females were near the stage of embryo release during all four winter cruises. During cruises 68–1 and 70–1, however, when female catch rates were highest, the proportion of females with eyed eggs was particularly high, and some fish (14.9% and 2.2%, respectively) were either spent or resting. Cruise 69–1 was undertaken after the spawning peak had been passed, and cruise 71–1 was made shortly before the spawning peak.

Table 3. Number of adults (36 cm and larger) caught per hour, by cruise. Data from the three major fishing depths at both sampling stations.

Cruise	Males	Females
	Summer cruises	
68–2	76	94
69–2	82	92
70–2	82	89
72–1	182	222
	Winter cruises	
68–1	93	223
69–1	76	30
70–1	77	130
71–1	46	63

Table 4. Percentage of mature females in each maturity stage during winter cruises, sampling stations combined. Maturity stages are: 3 = unfertilized; 4 = fertilized; 5 = with eyed embryos; 6 = spent or resting.

Cruise	Maturity stage 3	4	5	6	Number examined
68–1	1.8	1.2	82.2	14.9	342
69–1	.6	1.1	64.6	33.7	178
70–1		7.9	89.9	2.2	89
71–1		12.0	87.9		116

Differences Between Stations

There were no consistent differences between stations during summer cruises. Numbers caught per hour (depths combined) were greatest at the Quillayute station during 1968 and 1972 and at the Spit station during 1969 and 1970 (Table 2). Examination of the sex ratios encountered during summer cruises (Table 5) shows that males and females were found in approximately equal numbers.

During the winter cruises, however, females tended to be most predominant at the Quillayute station (especially at 200 fath) and catch rates were consistently higher there. This indicates that a geographic redistribution of fish occurs during the spawning period, with females concentrating at the 200-fath depth within the Quillayute station. The concentration of females at this station and depth was most evident during cruises 68–1 and 70–1 when the cruise dates coincided with the peak spawning period.

Size Composition

Differences Between Stations

Between-station differences were examined by comparing size compositions encountered at a

common depth. Comparisons were not made if fewer than 25 fish were caught per hour at one of the stations, or for the 160-fath series from cruise 69–2 (where deviation from the target fishing depth frequently exceeded 10 fath).

There were two modes in the size composition data from the shallow series (Fig. 2), one comprised of adult fish longer than about 30 cm and another of juveniles. The data indicated that when catch rates were low, the relative abundance of these two groups varied so widely between hauls that the data were unsuitable for analysis of a between-station effect. Catch rates for the shallow series were relatively high during cruises 68–2, 70–2, 72–1, and 69–1 (Table 2), so between-station differences were examined.

A X^2 test for homogeneity was made for each pair of size frequencies to be compared, and the

TABLE 5. Percentage of males encountered during each cruise, by station and depth.

Depth (fath)	Quillayute	Spit	Quillayute	Spit	Quillayute	Spit	Quillayute	Spit
Summer cruises	*68–2*		*69–2*		*70–2*		*72–1*	
120	49	47	53	50	51	43	54	53
160	52	56	55	52	55	54	50	54
200	41	38	43	52	58	46	38	43
All depths (weighted by CPUE)	48	48	50	52	54	47	50	52
Winter cruises	*68–1*		*69–1*		*70–1*		*71–1*	
150	80	58	80	68	60	48	47	67
200	23	53	45	86	27	64	37	78
250	68	41	53	67	62	59	66	47
All depths (weighted by CPUE)	31	45	68	74	33	61	43	52

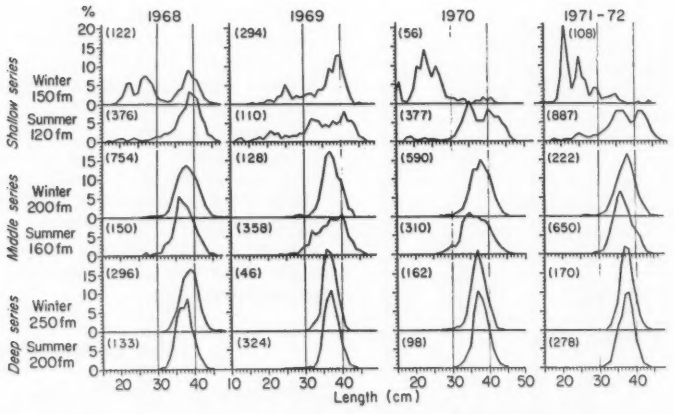

FIG. 2. Pacific Ocean perch (*Sebastes alutus*) size composition by year, cruise, and depth. (Number of fish caught per hour is shown in parentheses.)

results show that there were significant between-station differences in only 8 out of 18 of the comparisons:

Summer cruises			Winter cruises		
	X^2	df		X^2	df
120 fath			150 fath		
68–2	38.3	31	69–1	71.7[a]	28
70–2	46.1[a]	32			
72–1	151.2[a]	32			
160 fath			200 fath		
68–2	8.4	21	68–1	67.8[a]	26
70–2	22.8	23	69–1	9.4	21
72–1	70.7[a]	17	70–1	67.7[a]	22
			71–1	15.9	21
200 fath			250 fath		
68–2	15.9	14	68–1	63.5[a]	17
69–2	8.8	17	70–1	27.9[a]	16
70–2	17.5	14	71–1	9.8	12
72–1	18.4	12			

[a]Significant at the .05 level

Two of these significant X^2 values were associated with the concentration of spawning females at the Quillayute station, 200-fath depth during cruises 68–1 and 70–1. These concentrations were made up of much larger fish than were present at the Cape Flattery Spit station (Fig. 3), and analysis of age composition and age–length data for females shows that they were comprised of much older but slower growing fish than those at Cape Flattery Spit (Fig. 3, Table 6). Age and growth structure of males followed similar trends between stations, but the differences were not as great.

In general, the only time it was possible to detect size composition differences associated strictly with sampling station was during the peak spawning period. No consistent station effect could be demonstrated during other times of the year, and the effect was seldom strong enough to warrant separate treatment of the size composition data by station.

Differences Between Depths

Size composition data for each depth series have been summarized by cruise in Fig. 2. Within each cruise-depth stratum, the size composition data for each sex and sampling station have been weighted by the corresponding catch rate (numbers/hour) and combined.

In general, the results correspond to those of Westrheim (1970) who found that in the La Perouse area, juvenile fish were confined to the shallowest portion of the adult bathymetric range. The size composition of adults (those fish larger than about 30 cm) changed with depth, containing fewer fish from both the largest and smallest tails of the shallow-water length distribution as depth increased. This resulted in a relatively narrow range of sizes in the deepest portion of the bathymetric range and a sharp peak in the size frequency data from there.

During winter, the entire size composition array shifted downslope. This shift seemed to be quite uniform throughout the bathymetric range (Fig. 2) so that changes in size composition with depth followed the same patterns as in summer.

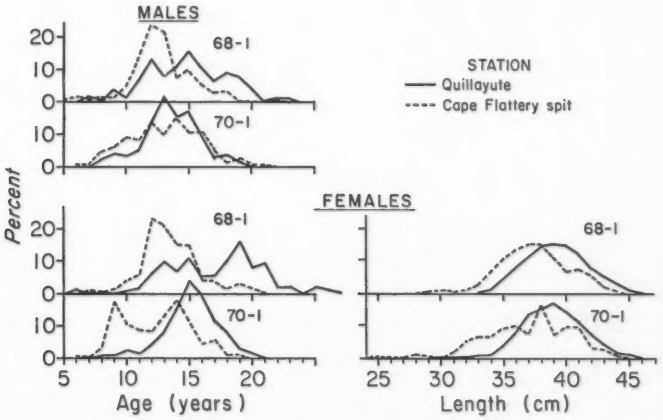

Fig. 3. Differences in age and size composition between sampling stations during the spawning peak, cruises 68–1 and 70–1, 200 fath.

TABLE 6. Mean length and sample size (parentheses) at each age during cruises 68–1 and 70–1, 200 fath.

	Catch rate (no./hour)	Age 6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
								Cruise 68–1 males									
Quillayute	300				31.8 (3)		34.5 (3)	35.5 (9)	35.7 (5)	36.3 (6)	35.8 (10)	36.0 (2)	36.0 (2)	37.3 (4)	36.0 (1)		
Spit	96	28.0 (2)		32.0 (2)	34.5 (2)	33.7 (6)	34.7 (16)	35.5 (27)	36.3 (28)	37.6 (7)	37.1 (10)	38.5 (6)		38.2 (3)			
								Cruise 68–1 females									
Quillayute	1,029	27.5 (2)					34.8 (3)	35.4 (7)	35.6 (8)	38.5 (9)	36.9 (13)	38.5 (5)	38.0 (4)	38.0 (6)	38.6 (15)	37.0 (4)	39.1 (9)
Spit	84				32.5 (2)	33.9 (5)	35.0 (6)	36.5 (23)	37.1 (25)	37.5 (15)	39.5 (16)	40.0 (4)			39.5 (3)	41.0 (2)	
								Cruise 70–1 males									
Quillayute	236			31.0 (2)	31.0 (7)	33.5 (6)	34.9 (7)	35.4 (21)	36.3 (32)	36.7 (23)	37.3 (26)	37.4 (16)	38.0 (5)	39.1 (7)	39.0 (2)		
Spit	186	27.0 (2)	29.0 (2)	31.7 (15)	33.2 (18)	34.1 (26)	36.1 (21)	36.6 (38)	38.0 (25)	38.7 (38)	39.9 (27)	40.2 (29)	40.5 (14)	42.0 (5)	41.6 (7)	42.0 (2)	
								Cruise 70–1 females									
Quillayute	651	30.0 (2)		31.3 (3)	32.5 (4)	34.5 (11)	35.2 (6)	35.9 (17)	36.8 (28)	37.9 (47)	38.9 (73)	39.6 (58)	40.6 (34)	40.1 (23)	41.9 (9)	41.2 (5)	
Spit	105	26.5 (2)		32.5 (6)	33.5 (31)	33.9 (19)	35.5 (15)	37.4 (14)	38.3 (23)	38.8 (32)	40.5 (21)	40.6 (8)	41.9 (7)	41.5 (4)			

Age–Length Relation

Differences Between Depths and Cruises

When comparing growth curves from several depths and cruises, and each curve is constituted from 10 to 15 mean length-at-age observations, a confusing mass of points and lines quickly results. To get around this difficulty, a least-squares technique (Tomlinson and Abramson 1961) was employed to fit observed data to the Bertalanffy growth model

$$l_t = L_\infty (1-e^{-K(t-t_0)})$$

where l_t = length of fish (cm at age t years), L_∞ = theoretical asymptotic length, K = constant expressing the rate of approach to L_∞, t_o = theoretical age at which $l_t = 0$.

All comparisons of the age–length relationship at different depths, seasons, and areas were then made by comparing fitted length at a common age. Growth differences between these strata seemed to increase with age, and since 15-year-olds were generally the oldest age-group for which age data were available from all sampling depths, this was taken as the reference age. Westrheim (1973) plotted bathymetric changes in mean length for several age-groups of Pacific Ocean perch from La Perouse bank, and his results indicate that these changes are of similar magnitude for all 14–20-year-olds. Bathymetric changes in the mean length of 12-year-olds were much smaller than those of the 14–20-year group.

Least-squares curves could be fitted only when data from a continuous series of ages were available, with no gaps occurring. It was necessary to combine age–length observations from different sampling stations in order to obtain series like this and get reliable results. Table 7 shows the age range and number of age–length observations used to fit the Bertalanffy function for each cruise-depth-sex

Table 7. Number of fish and age range involved in fitting growth data to the Bertalanffy model; and fitted length at age 15 by depth, sex, and cruise.

		Winter cruises				Summer cruises			
		68–1	69–1	70–1	71–1	68–2	69–2	70–2	72–1
					Males				
Shallow series	Ages fitted	4–21	3–18	2–16		3–20	2–18	3–20	2–21
	No. of fish	142	193	161		211	177	424	390
	Fitted length at age 15	39.7	40.9	40.2		40.0	40.8	40.3	39.8
Middle series	Ages fitted	6–18	7–19	6–20	4–19	7–18	4–18	6–19	8–20
	No. of fish	157	194	423	277	174	607	527	314
	Fitted length at age 15	36.9	37.4	38.6	38.5	38.3	39.4	39.1	37.9
Deep series	Ages fitted	9–20		8–20	9–18	9–19	7–19	9–20	8–22
	No. of fish	119		289	200	72	394	175	133
	Fitted length at age 15	36.8		37.4	37.8	37.4[a]	37.8	36.8	37.3
					Females				
Shallow series	Ages fitted	4–15				2–20	2–18	3–22	2–24
	No. of fish	66				226	267	570	353
	Fitted length at age 15	42.4				41.0	42.4	41.9	40.3
Middle series	Ages fitted	6–23	7–20	6–20	7–20	7–19	3–18	6–20	6–22
	No. of fish	193	215	504	290	165	697	485	287
	Fitted length at age 15	37.9	39.5	39.1	39.7	39.8	40.8	40.4	39.0
Deep series	Ages fitted	9–22		7–20	9–19	9–20	8–20	9–20	9–23
	No. of fish	65		189	118	123	651	129	206
	Fitted length at age 15	38.3		38.1	38.9	38.3	39.0	38.7	38.2

[a]Attempted to fit model, but failed. Figure given is actual mean length at age 15.

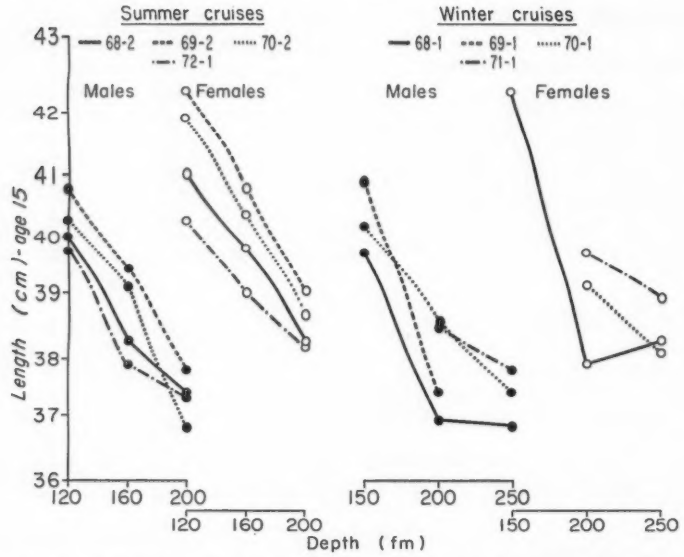

FIG. 4. Bathymetric changes in fitted lengths at age 15, by sex and cruise.

stratum, as well as the fitted length at age 15. Curves could not be fitted for some strata because there were too few age-length observations available.

The end results of this work are shown graphically in Fig. 4 and 5. Figure 4 shows that there were bathymetric changes in the age–length relation for each cruise with fitted length at age 15 consistently declining with increasing depth. During summer, the decline was almost linear in all years. During winter, however, bathymetric changes in the age–length relationship differed somewhat between cruises. Males from the 1970 winter cruise exhibited the linear summer pattern, while during 1968 the decline in fitted length leveled off between 200 and 250 fath.

Although the data in Fig. 4 were arranged to show bathymetric trends in growth, they also reflect the high within-depth variability in fitted length at age 15 that existed between cruises. Fitted length for females in the shallow depth series varied by as much as 2.1 cm between summer cruises. This variability is quite striking in a slow-growing species like *S. alutus*, especially when least square curves are being compared.

The change in fitted length at age 15 with depth is quite sharp (Fig. 4), and this phenomenon acting in conjunction with slight between-cruise differences in depth sampled or in the bathymetric distribution of the *S. alutus* stock probably accounts for much of the variability. Some additional between-cruise variability was caused by changes in growth characteristics with catch rate, however, (Fig. 5) since there was a tendency for fitted length at age 15 to

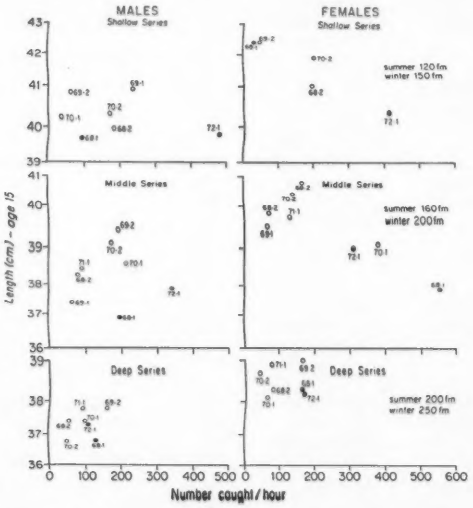

FIG. 5. Relationship between catch rate and fitted lengths at age 15, by sex, in three different portions of the bathymetric range. Cruises 68–1 and 72–1 are denoted by solid dots.

decline with increasing catch rate wherever data from a wide range of CPUE observations were available. For most depth series, fitted length at age 15 was quite low during cruises 68–1 and 72–1 (solid dots in Fig. 5) when maximum overall catch rates were encountered (Table 2).

Overall Age–Length Relation

Because of variation in the age–length relation between depths and cruises, growth curves representative of the entire S. *alutus* stock off northern Washington are difficult to obtain. Such curves are necessary in studies of population dynamics, especially if dynamic pool models are to be used. Consequently, an attempt was made to weight the growth data for different depths and seasons by the abundance of fish in that particular stratum and arrive at "overall" growth curves for *S. alutus* off the northern Washington coast. The computations were carried out for all 1968–71 cruise data, but were not updated after the 1972 cruise.

The relative position of the sampling depths within the *S. alutus* bathymetric range seemed to differ between summer and winter cruises, so data from these two sources were treated separately. Growth also differs between sexes, so the initial goal was to obtain four growth curves, one for each sex and season. To do this, age–length data for both sampling stations and all years in the summer and winter cruises were combined. The next step was to weight the mean length in each depth and age-group by the catch rate of *S. alutus* in that stratum and obtain mean length at age for summer and winter cruises (Fig. 6). As a final step, data from the summer series and winter series were combined again using a weighted mean for each age-group. These data were then fitted to the Bertalanffy growth model using the least-squares technique previously outlined. The estimated parameters are:

	Males	Females
L_∞	42.44	44.74
K	.146282	.128152
t_o	−1.1915	−1.5971

Calculated values of mean length at each age are shown in Fig. 6.

Age Composition

Between-depth and between-cruise variation in *S. alutus* age composition is portrayed in Fig. 7. Within each cruise-depth stratum, the age composition data for each sex and sampling station have been weighted by the corresponding catch rate (numbers/hour) and combined. Within the adult mode group (fish 11 years and older), no consistent pattern of variation was apparent upon cursory examination of Fig. 7, so analysis of variance was performed to test for age differences between years, cruises, and depths.

A nested design with 3 years (1968–70), two cruises within each year, and three depths within each cruise was used. Data for the two sampling stations were combined and the depths used were 120, 160, and 200 fath in summer and 150, 200, and

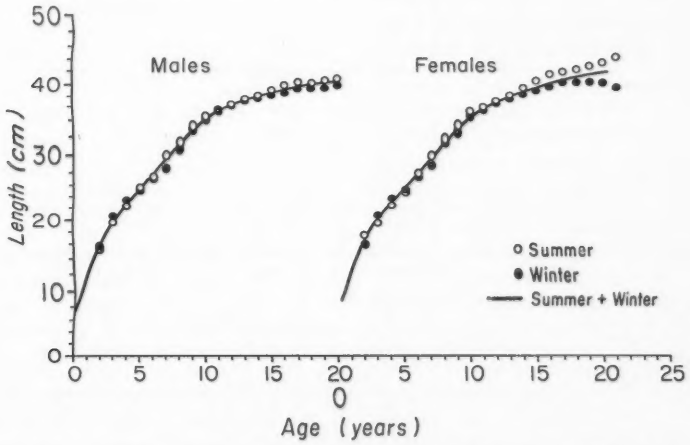

FIG. 6. Growth curves for Pacific Ocean perch off the northern Washington coast.

250 fath in the winter. Mean age of fish 11 years and older was used as the dependent variable in each cell and the analysis was carried out by using the BMD O5V computer program. The results are as follows:

Source of variation	Males df	Males F	Females df	Females F
Year	2	5.965[a]	2	12.812[a]
Cruise	1	.401	1	.034
Depth	2	.692	2	3.316
Year × cruise	2	1.802[b]	2	5.183[a]
Year × depth	4	1.231[b]	4	2.332[b]
Cruise × depth	2	.030[b]	2	4.979[a]
Error	4		3[c]	

[a]Significant at the .05 level.
[b]Not significant at the .20 level, pooled with error term.
[c]Data were insufficient to include a value for mean age at the 150-fath depth, 1970 winter cruise. This resulted in the loss of 1 df in the error term.

Results for both males and females indicate that age composition differences between depths and seasons (cruise effect) were nonsignificant, but that there was a significant year effect.

The failure to detect a significant between-depth difference in mean age indicates that adults within different portions of the bathymetric range have a similar age composition, and the bathymetric changes in length composition seen in Fig. 2 are caused mainly by the inverse relationship between mean length at a given age and depth.

Age composition data for each depth series were also weighted by the appropriate catch rate and combined by cruise. The results (Fig. 8) show that the most obvious changes in age composition took place during cruises 68–1 and 72–1 when a high proportion of fish 16 years and older was caught. These were the cruises where peak catch rates and low values for fitted length at age 15 were encountered, and considering all available evidence, it seems that there was a sharp increase in the availability of a group of old, slow-growing fish during these periods. The effects of this increased availability are manifested in the analysis of variance, being at least partially responsible for the significant year × cruise and cruise × depth interactions that were found in the analysis for females. Until several intraseasonal comparisons have been made and we can be certain that such changes in availability are not short-term phenomena, no firm conclusions can be made concerning between-year changes in adult age composition.

There were substantial changes in the age composition of juveniles (fish less than 11 years old) with depth, young fish being most abundant in the shallowest part of the bathymetric range. The data for most cruises (Fig. 7) showed sharp reductions in the percent contribution of 2–5-year-olds be-

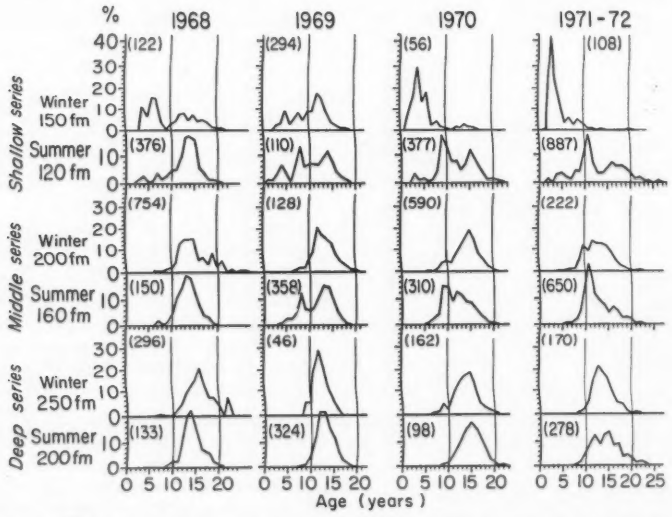

FIG. 7. Pacific Ocean perch age compositions by year, cruise, and depth. Number of fish caught per hour is shown in parentheses.

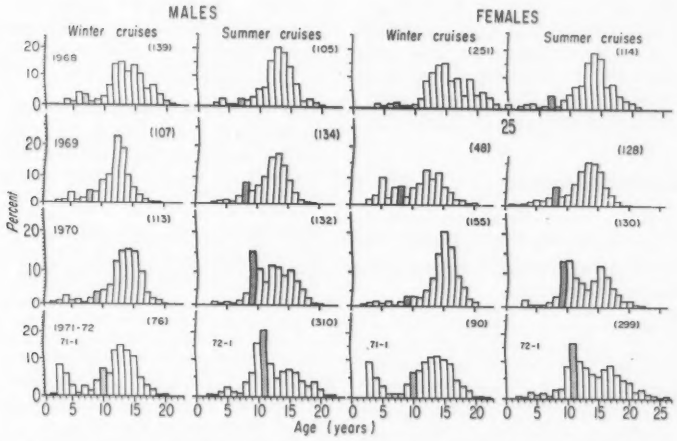

FIG. 8. Pacific Ocean perch age compositions by year, cruise, and sex; depths combined. Number of fish caught per hour is shown in parentheses and stippled areas denote the 1961 class.

tween the shallow and middle series and of 5–9-year-olds between the middle and deep series.

The age composition of juveniles also differed between years, owing to the dominance of the 1961 year-class. Juveniles were generally most abundant during summer cruises and data from those cruises show the progression of this year-class best. Both males and females show modes at 7 years in 1968, 8 years in 1969, 9 years in 1970, and 11 years in 1972 (Fig. 8).

Conclusions

Westrheim (1973) found an inverse relation between depth and growth rate for fish off southwest Vancouver Island and concluded that the disjunction in age–length relationship between shallow and deep-water aggregations was sharp enough to consider them as separate subpopulations. Data from the present study tend to support this conclusion since intermixing between shallow- and deep-water fish was never significant enough to disrupt the pattern of growth decrease with depth (Fig. 4). Most fish 10 years and younger are found in the shallow and intermediate portion of the bathymetric range (Fig. 7), indicating that segregation of recruits into shallow water and deep-water components doesn't occur until age 11. This explains why adult age compositions did not vary much with depth, since only a limited amount of time is available for intra-population differences in mortality to generate differences in age composition.

The most unique outcome of the cruises was the detection of a relatively old, slow-growing segment of the *S. alutus* population that varied widely in its availability to on-bottom trawl gear. Data on catch rates (Table 2 and 3) and sex ratios (Table 5) indicate that male and female components of this group were equally available during the 1972 summer cruise, but that it was mostly the female component that was available during periods of peak spawning (cruises 68–1 and 70–1). At present, it is not possible to say whether this segment of the *S. alutus* population is present in the study area at all times but only occasionally aggregates on the bottom or whether they are usually found outside the study area.

Only by broadening the sampling program so that a wider variety of sampling stations are covered and these stations are sampled at several times of the year will we determine which alternative is most realistic, and in either case formidable problems face any worker who is monitoring changes in abundance. Unless it is possible to predict changes in availability (i.e. by monitoring oceanographic changes if this is the decisive factor), acoustical gear capable of locating and enumerating all segments of the population will be a virtual necessity. In the absence of such gear, the only alternative is to broaden the sampling program in time and space to make indices of abundance as insensitive as possible to short-term changes in availability.

In the case of *S. alutus*, age composition fluctuates with changes in availability, making it even more important that all segments of the population are

located and sampled in proportion to their abundance. Otherwise, estimates of mortality could be made only between those cruises where the old, slow-growing component of the population was equally available and any program of monitoring changes in mortality would be relatively ineffective.

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Behavioral and Developmental Plasticity of Buoyancy in the Longnose, *Rhinichthys cataractae*, and Blacknose, *R. atratulus* (Cyprinidae) Dace

JOHN H. GEE

Department of Zoology
University of Manitoba, Winnipeg, Man.

GEE, J. H. 1974. Behavioral and developmental plasticity of buoyancy in the longnose, *Rhinichthys cataractae*, and blacknose, *R. atratulus* (Cyprinidae) dace. J. Fish. Res. Board Can. 31: 35–41.

The hypothesis that developmental plasticity contributes to variation in swimbladder length, volume, weight of tissue, and buoyancy was examined in two species of dace. At both maximum and minimum buoyancy attained dace reared in still water to adult size possessed swimbladders of a greater length, volume, and weight of tissue than those reared in current. Such developmental plasticity affected the range over which buoyancy could be adjusted (behavioral plasticity). Those reared in still water attained a more buoyant condition than those reared in current while the latter attained a greater degree of negative buoyancy.

GEE, J. H. 1974. Behavioral and developmental plasticity of buoyancy in the longnose, *Rhinichthys cataractae*, and blacknose, *R. atratulus* (Cyprinidae) dace. J. Fish. Res. Board Can. 31: 35–41.

Nous examinons chez deux espèces de naseux l'hypothèse que la plasticité du développement contribue à des variations de la longueur, du volume, du poids du tissu de la vessie gazeuse et de la flottabilité. Tant au maximum qu'au minimum de flottabilité, les naseux élevés en eau morte jusqu'à la taille adulte possèdent une vessie gazeuse d'une longueur, d'un volume et d'un poids tissulaire plus grands que celle des poissons élevés dans un courant. Une telle plasticité de développement affecte l'intervalle dans lequel la flottabilité peut être ajustée (plasticité de comportement). Les naseux élevés en eau morte atteignent un degré de flottabilité plus élevé que les poissons élevés dans un courant, alors que ces derniers atteignent une plus grande flottabilité négative.

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BOTH genotypic and phenotypic plasticity enable species to cope with changes in their environment (Birch 1960). The former is involved with changes occurring over periods greater than the life-span of the individual, the latter with changes within the life-span of an individual. Phenotypic plasticity can be of two types: behavioral and developmental (Thoday 1953). Developmental plasticity allows individuals to develop different but adaptive phenotypes in different environments while an individual possesses behavioral plasticity if it can adapt temporarily to sudden changes in its environment. This paper seeks to determine if developmental plasticity contributes to variation in swimbladder volume and length, and buoyancy found within two species of stream-dwelling minnows.

Within populations of longnose and blacknose dace (*Rhinichthys cataractae*, *R. atratulus*), and between populations of the former, the considerable variation in length and volume of the swimbladder relate to velocity of current in the environment (Gee 1972). Laboratory experiments have shown that both species exhibit behavioral plasticity by adjusting swimbladder volume and length rapidly to alter buoyancy in response to changes in water velocity (Gee 1968, 1970, 1972). Such plasticity does not account for all the variation found in swimbladder volume or length within and between populations of longnose dace and evidence suggests the occurrence of developmental plasticity (Gee 1972). The hypothesis tested here is that the development of the swimbladder in terms of length, volume, and weight of tissue is influenced by the speed of current in which the fish is reared. Such plasticity would affect the range over which buoyancy could be adjusted.

Plan of Experiment

Blacknose and longnose dace from the Mink River, Manitoba and longnose dace from Lake Winnipeg were examined for developmental plasticity. Recently emerged

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fry (<20-mm fork length) were collected and taken to the laboratory where one half of the fry from each population was reared in still water, the other in current. The effect of rearing in still water or current on swimbladder length, volume, weight of tissue, and buoyancy attained was examined at both maximum (attained in still water) and minimum (attained in current) buoyancy.

Periodically a sample of eight fish from each population was taken from rearing tanks with current, held for 7 days in still water to reach maximum buoyancy and compared to eight fish reared throughout their captive lives in still water. A similar comparison was made of minimum buoyancy by taking fish reared in still water, holding them in current for 7 days and comparing them to those reared in current.

Materials and Methods

Dace reared in current were held in glass aquaria (122 × 61 × 61 cm). Depth of water was 45 cm. Current was created by bubbling compressed air through air

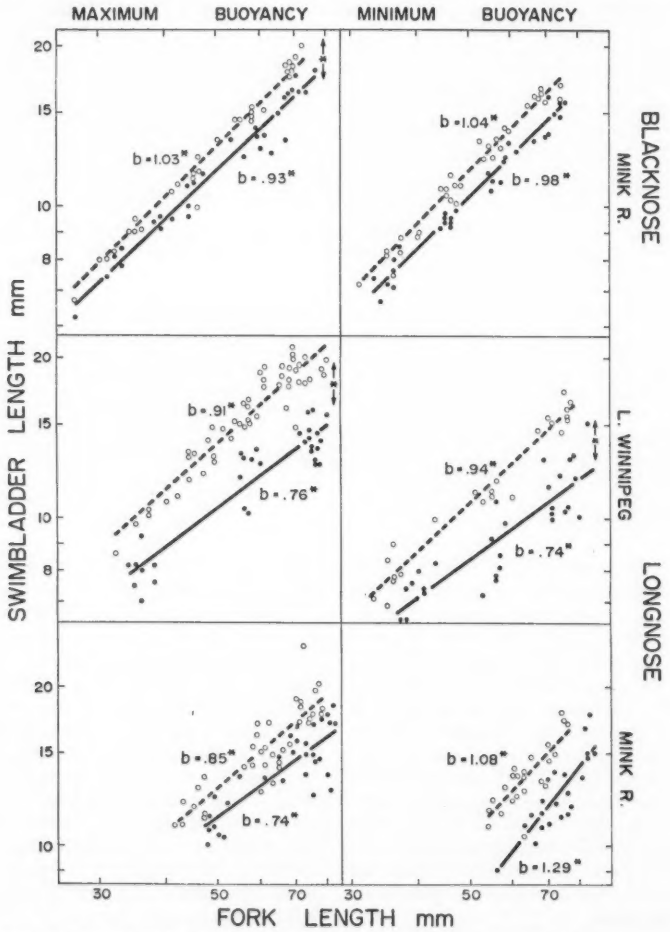

FIG. 1. Swimbladder length of dace reared in still or current at maximum and minimum buoyancy. Regression lines for fish reared in still (open circles) are broken; those for fish reared in current (solid circles) are unbroken. Asterisks next to slopes indicate significant ($P < 0.05$) regressions. Asterisks between two arrows indicate significant difference between slopes ($P < 0.05$) and where absent there are significant differences between intercepts ($P < 0.05$).

diffusor bars on the bottom at one end creating a circular current on a vertical plane. Stones (2–12 cm diam) provided crevices for dace and produced a turbulent flow of water. Velocity 1 cm above the substrate varied spatially from 23 to 45 cm/sec.

Dace reared in still water were held in glass aquaria (92 × 46 × 46 cm) with a small air diffusor stone gently bubbling at the surface. Water depth was 38 cm. Similar aquaria were used to hold dace in still water for the 7 days prior to examination to attain maximum buoyancy. But dace held 7 days in current to attain minimum buoyancy were contained in a stainless steel screen cage (41 × 60 cm) with a plexiglass bottom and suspended in an aquarium in 5 cm of water flowing at 45–50 cm/sec

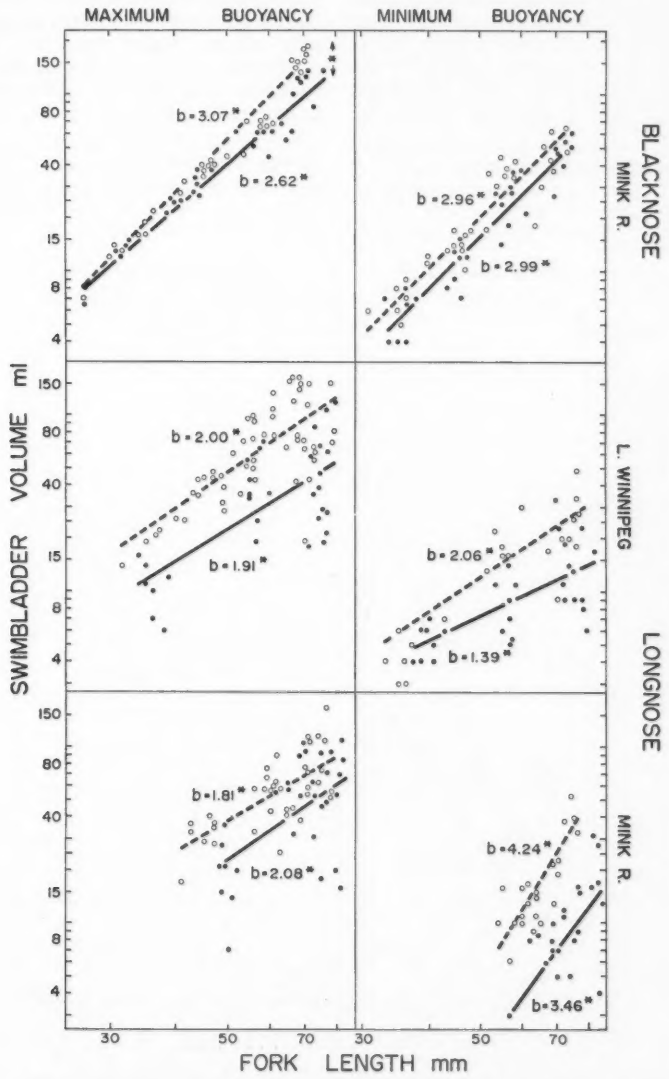

FIG. 2. Swimbladder volume of dace reared in still or current at maximum and minimum buoyancy. Symbols and explanation as in Fig. 1.

(Gee and Bartnik 1969). Flat stones on the bottom of the cage provided a more turbulent flow.

In the laboratory dace were kept at 21 C (± 1) under a 12-hr photoperiod and fed frozen brine shrimp and Tetramin flakes.

On examination, a dace was captured and pithed. Swimbladder volume was determined by subtracting the weight of the fish in water with its swimbladder intact from the weight of the gas-free-fish in water. This difference in weight (± 0.001 g) equals the volume of the swimbladder (± 0.001 ml). Swimbladder length (± 0.1 mm) was measured from the intact, inflated bladder. Weight (± 0.001 g) of swimbladder tissue, removed and dried lightly on blotting paper, was measured on larger dace. Flotation pressure, a measure of buoyancy (Saunders 1953), was obtained by dividing swimbladder volume by the weight of the gas-free-fish in water (1.0 ml/g = neutral buoyancy; < 1.0 = negative buoyancy).

Results

At maximum and minimum buoyancy dace reared in still water had longer swimbladders than those reared in current (Fig. 1). This difference was greatest among larger fish with blacknose dace at maximum buoyancy and longnose dace from Lake Winnipeg at maximum and minimum buoyancy.

Dace reared in still water possessed swimbladders with a larger mean volume than those reared in current except for blacknose dace at maximum buoyancy (Fig. 2). Here differences existed only among larger fish.

Weight of swimbladder tissue from dace over 65-mm fork length was greater for fish reared in still water than in current (Fig. 3).

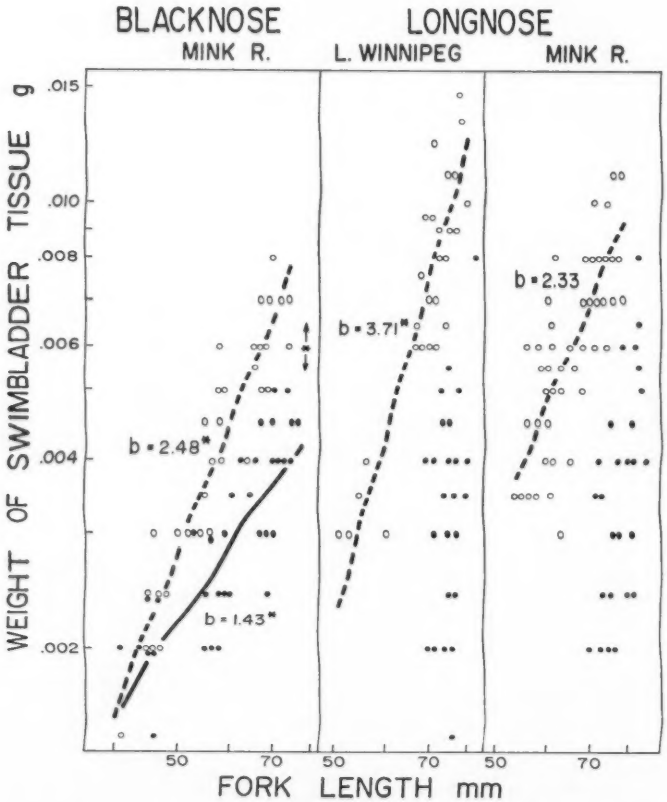

FIG. 3. Weight of swimbladder tissue of dace reared in still or current. Symbols and explanation as in Fig. 1. Regression lines were not fitted to some data because of restricted ranges in length of some populations examined.

Buoyancy, as measured by flotation pressure was influenced by the environment in which dace were reared. At maximum buoyancy fish reared in still water had a higher flotation pressure than those reared in current although differences were dependent on length as indicated by different slopes of regression lines (Fig. 4). The greatest differences in flotation pressures between fish reared in still water and current were found among the largest blacknose dace and the smallest longnose dace. At minimum buoyancy dace reared in still water had a higher flotation pressure than those reared in current, and differences were independent of length.

A comparison of the effect of behavioral (adjustment of buoyancy) and developmental plasticity on variation in buoyancy can be made by using regression lines for flotation pressures of dace reared in still or current at maximum and minimum buoyancy. Total variation in flotation pressure is considerable in both species. Among blacknose dace it is similar in all sizes while in longnose dace it is less for larger fish (Fig. 5). Both behavioral and developmental plasticity contribute to this variation. Except for blacknose dace reared in still water, behavioral plasticity was greatest among smaller dace and decreased for larger fish of both species. Developmental plasticity at maximum buoyancy was greatest among larger blacknose dace and smaller longnose dace. At minimum buoyancy there was no variation with size in either species.

Discussion

Both species show developmental plasticity in swimbladder length, volume, and weight of tissue

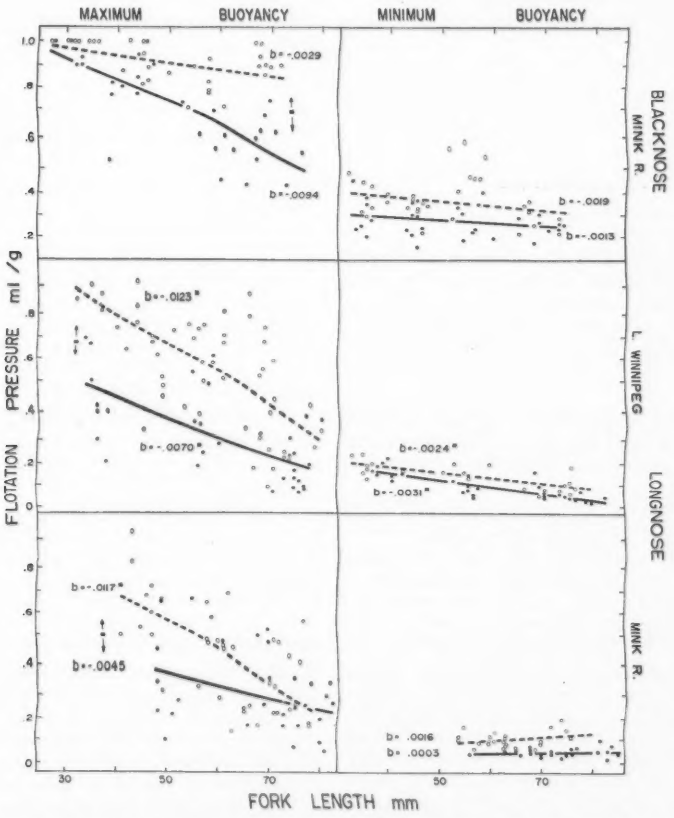

FIG. 4. Flotation pressure of dace reared in still or current at maximum and minimum buoyancy. Symbols and explanation as in Fig. 1.

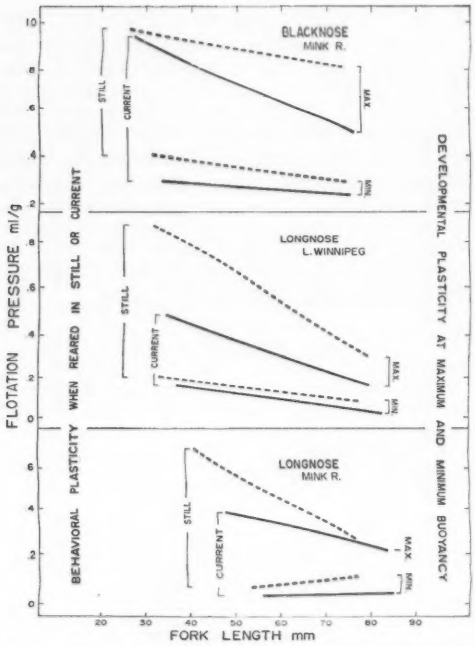

FIG. 5. Comparison of the effect of behavioral and developmental plasticity on buoyancy using regression lines for flotation pressure of dace reared in still (broken lines) or current (solid lines) at maximum and minimum buoyancy. Behavioral plasticity is represented by the difference between pairs of either solid or broken lines; developmental plasticity by the difference between upper or lower pairs of lines for each population.

that is determined by the velocity of water in which they are reared. Such plasticity affects the range over which buoyancy can be adjusted (behavioral plasticity).

The ability to adjust buoyancy and the range over which buoyancy is altered by both species relates well to places in which they live. Fry (< 30-mm fork length) of both species from the Mink River can attain neutral buoyancy. But blacknose fry cannot adjust buoyancy although longnose dace are capable of making their greatest adjustment at the fry stage (Gee 1972). In the Mink River blacknose dace occupy still waters in stream margins for their 1st year, then most move into moderate current (< 45 cm/sec) at lengths between 30–45 mm. Longnose dace also occupy still stream margins but only for about 3 weeks after which most move into fast-flowing riffles (> 45 cm/sec) at lengths between 25–30 mm (Gibbons and Gee 1972). This segregation in environment occupied is maintained throughout life, including reproduction (Bartnik 1970).

The lower minimum buoyancy attained by juvenile and adult longnose dace and the higher maximum buoyancy in blacknose dace relates well with this segregation. Within each species those individuals collected in the Mink River from faster water velocities were less buoyant than those from slower currents (Gee 1972). In Lake Winnipeg longnose dace occupy shallow onshore waters of wave-washed rocky beaches only in July and August and those collected on rough windy days were less buoyant.

Thus behavioral adjustments of buoyancy help the individual cope with sudden changes in current or turbulence that occur in streams and lakes over time and space. Developmental plasticity molds the phenotype of the individual over its life-span to cope with the long-term conditions of current encountered.

The ecological and evolutionary significance of developmental and behavioral plasticity is related to the resulting potential to occupy a variety of environments in terms of current or turbulence. Such plasticity could be one of the factors responsible for the present distribution of longnose and blacknose dace. At the same time it could contribute indirectly to their genetic diversity through the following sequence of events: (1) extension of distribution to exploit new and different environments, (2) isolation in new and different environments, and (3) genetic differentiation.

Freshwaters and their drainages are seldom permanent over long periods of time and opportunities for expansion of distribution and isolation are common for freshwater fishes. This combined with a variety of environments present in freshwaters sets the stage for isolation and genetic differentiation between populations. Blacknose dace and in particular longnose dace are capable of occupying a variety of environments (Nichols 1916; Gee 1968; 1972; Gee and Northcote 1963; Gee and Machniak 1972; Gibbons and Gee 1972). The present distribution of both species, especially longnose dace, is broad and has expanded considerably since the Wisconsin Glaciation (Trautman 1957; Hubbs and Lagler 1964; McPhail and Lindsey 1970). Blacknose dace is represented by four subspecies (Hubbs and Lagler 1964). Longnose dace forms a number of geographic races whose subspecific status has not been clearly defined (McPhail and Lindsey 1970) although four subspecies have been described (Nichols 1916; Hubbs and Lagler 1964; J. D McPhail personal communication). Behavioral and developmental plasticity have undoubtedly contributed directly to the expansion of distribution of longnose and blacknose dace and indirectly to their evolutionary change.

Acknowledgments

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Lepeophtheirus cuneifer sp. nov. (Copepoda: Caligidae), a Parasite of Fishes from the Pacific Coast of North America

Z. KABATA

Department of the Environment
Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C.

KABATA, Z. 1974. *Lepeophtheirus cuneifer* sp. nov. (Copepoda: Caligidae), a parasite of fishes from the Pacific coast of North America. J. Fish. Res. Board Can. 31: 43–47.

Lepeophtheirus cuneifer sp. nov. (Copepoda: Caligidae) is described and illustrated. This copepod is a parasite of marine fishes off the coast of Alaska, its hosts including *Raja binoculata* and *Hexagrammos lagocephalus*. Other possible hosts are *Leptocottus armatus* and *Theragra chalcogramma*.

KABATA, Z. 1974. *Lepeophtheirus cuneifer* sp. nov. (Copepoda: Caligidae), a parasite of fishes from the Pacific coast of North America. J. Fish. Res. Board Can. 31: 43–47.

L'auteur décrit, avec illustrations, *Lepeophtheirus cuneifer* sp. nov. (Copepoda: Caligidae). Ce copépode est un parasite de certains poissons marins du large de la côte d'Alaska, dont *Raja binoculata* et *Hexagrammos lagocephalus*. D'autres hôtes possibles sont *Leptocottus armatus* et *Theragra chalcogramma*.

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My attention was drawn recently to a small collection of parasitic Copepoda collected from marine fishes of southeastern Alaska and Prince William Sound by the Marine Biological Expedition of the National Museum of Canada (Bousefield and McAllister 1962). Among several species of *Lepeophtheirus* in that collection, one, collected in June 1961, proved to be still undescribed. Fishes on which it was found range also off the coast of British Columbia. The account of this species is presented below as a possible addition to the previously published account of the genus *Lepeophtheirus* in this region (Kabata 1973). It is patterned largely after that account, particularly in regard to selection of morphological characters with taxonomic significance.

Description

RECORD OF SPECIMENS: (i) adults — 4 females and 11 males; juveniles — 14 females and 1 male, taken in Tee Harbour, Lynn Canal (58°26′N, 134°47′W); (ii) 14 females and 1 male taken in Thistle Cove, Dixon Harbour (58°22′N, 136°52′W); (iii) 1 female taken 1½ miles south of Lituya Bay (58°30′N, 137°38′W) and (iv) 1 female taken in Yakutat Harbour. One adult female from locality (i) becomes the holotype of the species and is preserved in the National Museum of Natural Sciences in Ottawa, Reg. No. NMC 15454; one male from the same locality becomes the allotype (NMC 15455). The remaining specimens become paratypes (NMC 15456).

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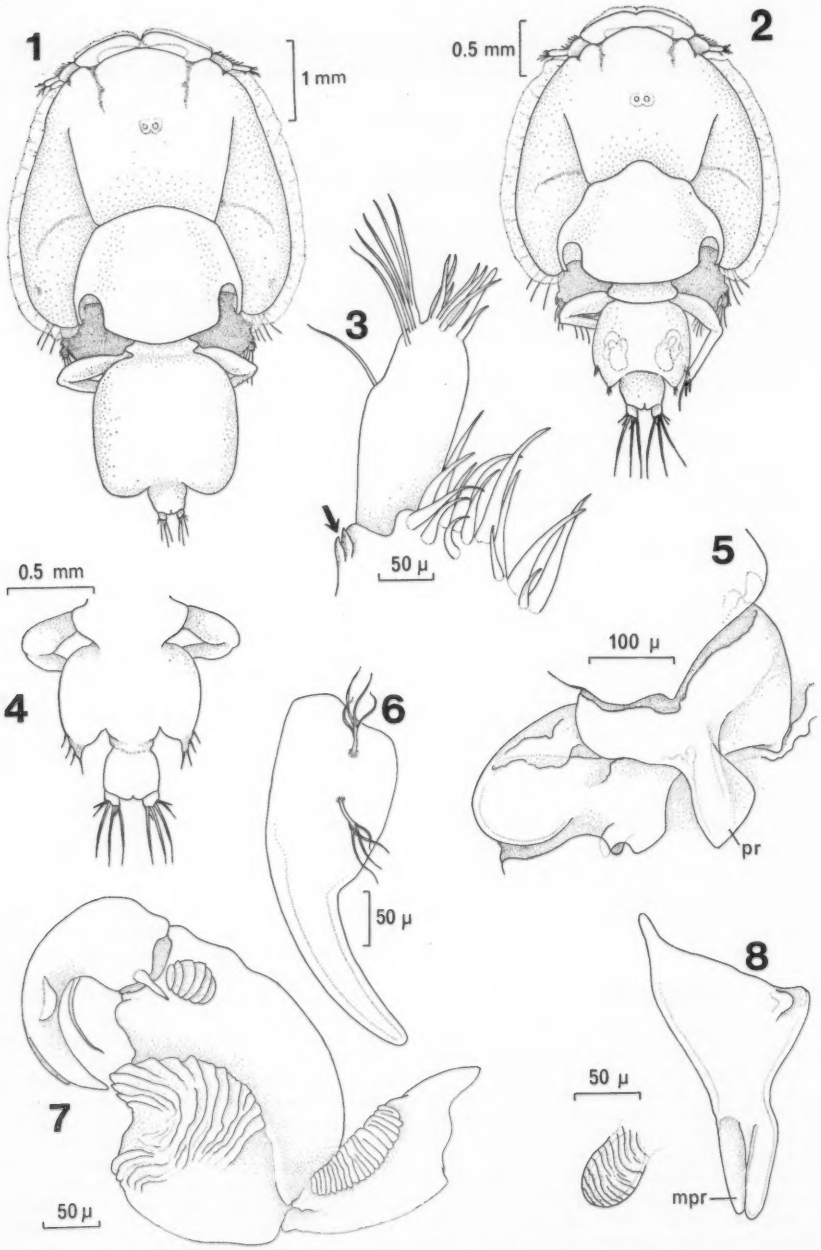
HOSTS: The collectors stored all copepods taken at one station in a single vial, so that it was not always possible to determine from which host any particular specimen came. At the type locality, *Leptocottus armatus* Girard 1854 and *Theragra chalcogramma* (Pallas 1811) were taken, so that either of these fish could have been the type host species. Two definitely identifiable hosts are *Raja binoculata* Girard 1854 and *Hexagrammos lagocephalus* (Pallas 1810). Specimens were also present in two vials containing parasites from *Isopsetta isolepis* (Lockington 1880), *Hippoglossus stenolepis* Schmidt 1904, *Gadus macrocephalus* Tilesius 1810, *Microgadus proximus* (Girard 1854), *Lumpenus sagitta* Wilimovsky 1956 and *Squalus acanthias* L.

Female (*Fig. 1*) — Dorsal shield oval, slightly ovate, tips of first antennae not extending to its lateral limits, posterior sinuses shallow, free margin of thoracic zone projecting only slightly beyond posterior tips of lateral zones. Fourth pedigerous segment about one-half width of posterior margin of shield, much shorter than wide, indistinctly delimited from genital complex. Latter about as long as thoracic zone of shield, with about equal length and width or somewhat wider than long, with sloping anterolateral corners, parallel lateral margins, rounded posterolateral corners and re-entrant posterior margin. Abdomen not clearly delimited from genital complex, one-segmented, about one-quarter length of genital segment, usually slightly broader at base, approximately equally long and wide. Dimensions of holotype specimen (in mm): total length (without frontal plates and caudal setae) 5.72, cephalothorax length 3.48, width 3.40, genital complex length 1.80, width 1.92, abdomen length 0.48, width (at base) 0.56, egg sac length 4.60, diameter 0.40.

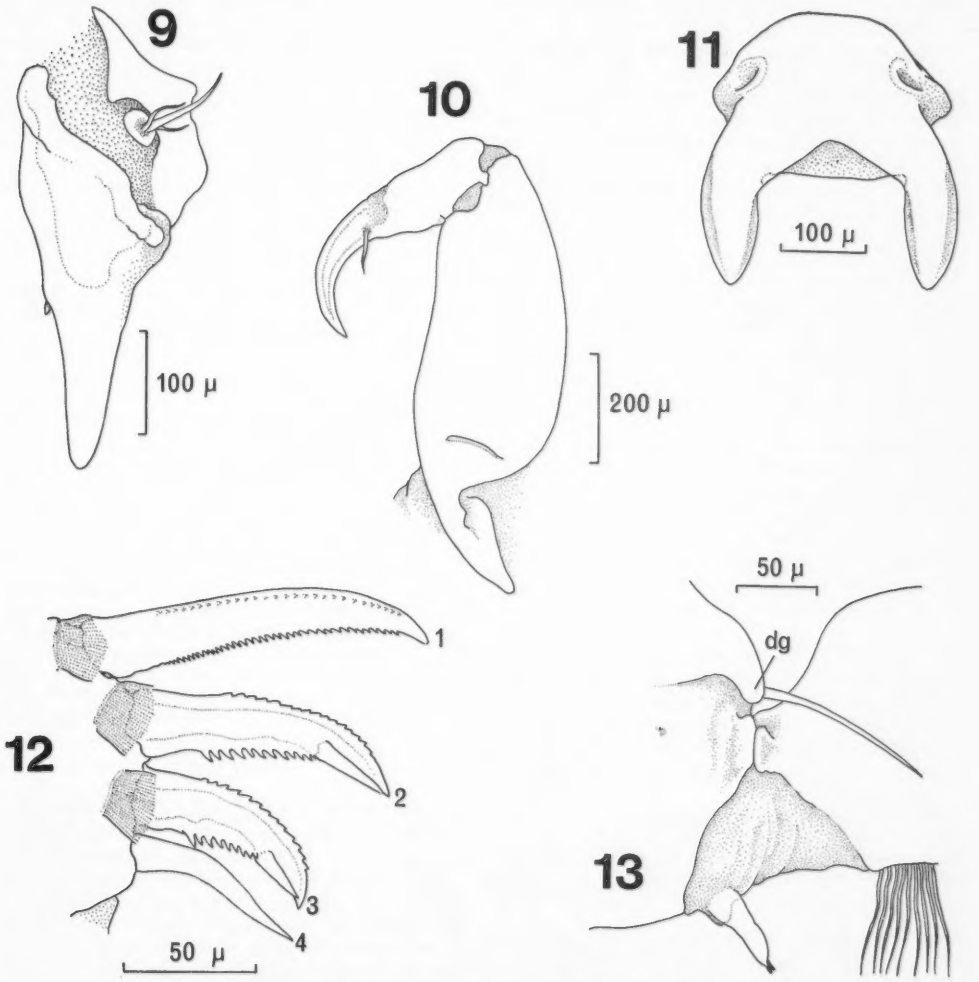


FIGURES 1–8. *Lepeophtheirus cuneifer*, female and male.

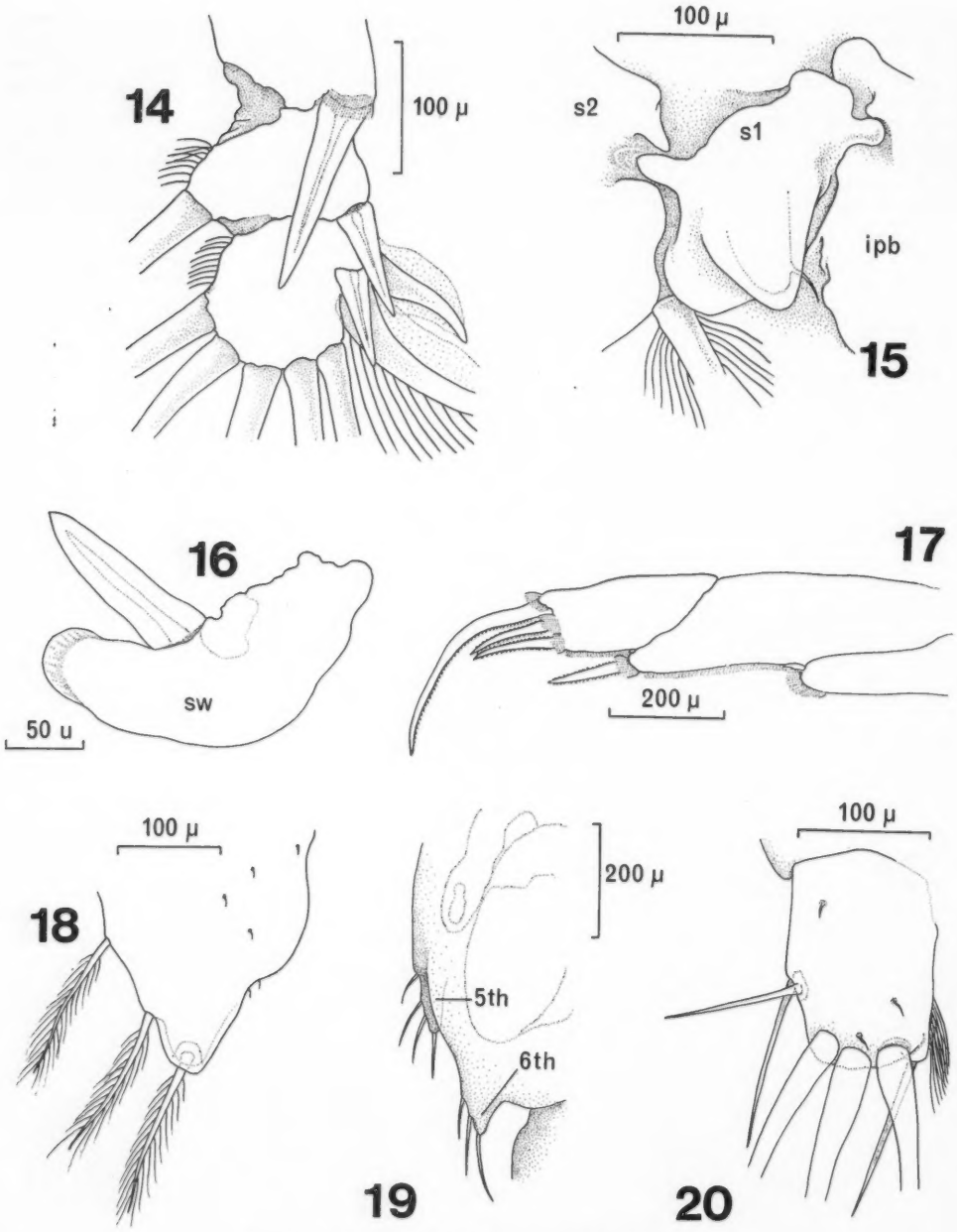
FIG. 1. Female, entire, dorsal; FIG. 2. Male, entire, dorsal; FIG. 3. Female and male, first antenna, distal half, ventral; FIG. 4. Juvenile female, posterior part, dorsal; FIG. 5. Female, base of second antenna, ventral; FIG. 6. Female and male, postantennary process, ventral; FIG. 7. Male, second antenna, posterior; FIG. 8. First maxilla and adhesion pad, ventral. (pr — posterior process of second antenna; mpr — medial process of male first maxilla.)

First antenna (Fig. 3) short, with broad basal segment. Posterodistal corner of latter with two sharp, flat processes (marked with arrow in Fig. 3). Second antenna of usual structure, posterior process (pr, Fig. 5) of second segment spatulate. Postantennary process (Fig. 6) with robust base about as long as tine, distally slightly tapering and curving. Dentiform process of first maxilla (Fig. 9) with single tine, broad at base and tapering towards blunt tip. At about midlength minute denticle present on medial margin. Maxilliped (Fig. 10) with narrow, slender corpus with transverse flange on ventral wall near base. Subchela with long, sharply curving claw. Sternal furca (Fig. 11) with box broader than long; tines widely spaced, divergent near base, parallel distally, with flanges on both margins.

Terminal armature of first exopod (Fig. 12) progressively shorter from seta 1 to seta 4. Seta 1 simple, with two rows of denticles, setae 2 and 3 with secondary processes in distal quarter and serrated on both anterior and posterior margins, seta 4 shorter than seta 1, simple



FIGURES 9–13. *Lepeophtheirus cuneifer*, female and male.
FIG. 9. Female, first maxilla, ventral; FIG. 10. Female and male, maxilliped, ventral; FIG. 11. Sternal furca, ventral; FIG. 12. First leg, terminal armature of exopod, ventral; FIG. 13. Same, joint between sympod and exopod, ventral. (dg — digitiform process.)



FIGURES 14–20. *Lepeophtheirus cuneifer*, female and male.

FIG. 14. Second leg, tip of exopod, ventral; FIG. 15. Same, first segment of sympod, ventral; FIG. 16. Third leg, basal spine of exopod, ventral; FIG. 17. Fourth leg, exopod, ventral; FIG. 18. Female, fifth leg, ventral; FIG. 19. Male, posterolateral corner of genital complex, ventral (with fifth and sixth legs); FIG. 20. Female and male, caudal ramus, ventral. (ipb — interpodal bar; s1, s2 — first and second segment of sympod; sw — basal swelling of third exopod.)

and pinnate (pinnules omitted in Fig. 12). Joint between sympod and exopod of first leg (Fig. 13) with digitiform process (dg) covering base of seta. First segment of sympod of second leg (s1, Fig. 15) bearing conical protuberance armed with single setule. Lateral spines of second exopod (Fig. 14) moderately short, that of distal segment barely reaching outer limit of segment. Basal spine of third exopod (Fig. 16) some distance from tip of basal swelling (sw); latter with terminal flange and two or three setules on lateral margin (variable, omitted in Fig. 16). Exopod of fourth leg (Fig. 17) three-segmented, with three terminal setae, first seta more than twice length of other two, second and third about equally long, all three with basal pectens. First seta with one serrated margin, second and third with two such margins. Seta of middle segment similar to preceding one, that of first segment vestigial, unciform, with smooth margins. Fifth leg (Fig. 18) subtriangular, not projecting beyond margins of genital complex. Caudal ramus (Fig. 20) about one-quarter length of abdomen, subrectangular, with distal half of lateral margin sloping inwards.

Male (*Fig. 2*) — Cephalothorax as in female. Genital complex shorter than thoracic zone of shield, only slightly wider than fourth pedigerous segment, with re-entrant posterior margin. Fifth and sixth legs visible in dorsal aspect. Dimensions of paratype specimen (in mm): total length 3.60, cephalothorax length 2.32, width 2.32, genital complex length 0.88, width 0.84, abdomen length 0.32, width 0.44.

Second antenna (Fig. 7) with inflated middle segment, larger adhesion pad moderately well developed, with smooth central area and low ridges. Terminal claw simple, with strongly developed base and tapering, sharply curving tip. Semicircular flanges on either side of claw, at midlength (only one visible in Fig. 7), outer margin of distal half with medial, longer, narrower flange. Short, robust seta at base of claw, long slender seta some distance from it, on concave margin. Dentiform process of first maxilla (Fig. 8) with single tine and low ridge on its proximal part; medial process (mpr) as long as, or slightly shorter than, tine. Oval, transversely ridged adhesion pad on ventral surface of cephalothorax medial to distal half of process. Fifth leg (Fig. 19) narrow, digitiform, with three setae on lateral margin and fourth at base. Sixth leg of three setae on lateral margin of subtriangular process (Fig. 19).

Comments

To establish the identity of the species described above, I had to compare it with all those members of the genus *Lepeophtheirus* that have abdomina shorter than one-half length of the genital complex and the dentiform process of the first maxilla either with a single tine or with the second tine greatly reduced. Thirteen such species had to be taken into account. Significant difference in at least one of the diagnostically important characters, as selected in a previous publication (Kabata 1973), was accepted as evidence that the compared species are not identical with *L. cuneifer*. The structure of the fourth leg differentiates between *L. cuneifer* and nine of these species (*L. acutus* Heegaard 1943, *L. bonaci* Pearse 1952, *L. christianensis* Wilson 1944, *L. clarionensis* Shiino 1959, *L. dissimilatus* Wilson 1905, *L. distinctus* Hewitt 1963, *L. longispinosus* Wilson 1908, *L. orbicularis* Shiino 1965, and *L. yanezi* Stuardo and Fagetti 1961). The structure of the fifth leg distinguishes between it and *L. spinifer* Kirtisinghe 1937, that of the fifth and third from *L. nanaimoensis* Wilson 1912 and that of the abdomen from *L. cossyphi* Krøyer 1863. The unusual, trifid structure of the tip of the second maxilla of *L. marginatus* Bere 1936 excludes the possibility of specific identity between it and *L. cuneifer*.

Three other species were considered, all with short abdomina but with unknown structure of the first maxilla. *L. brachyurus* Heller 1865 differs from *L. cuneifer* by the structure of its fourth leg, *L. plectropomi* Nunes-Ruivo and Fourmanoir 1956 and *L. plotosi* Barnard 1948 by the structure and size of their fifth legs.

Possessing both a short abdomen and the dentiform process of the first maxilla with a single tine, and yet being distinct from the above 16 species, the specimens here described have to be assigned to a new taxon. The name proposed for it refers to the wedge-shaped processes of the first maxillae and to the protuberances on the sympod of the second legs. The latter structure has not been previously seen by me in any species of *Lepeophtheirus* and might become an excellent diagnostic feature of *L. cuneifer*. Unfortunately, its apparent absence from other species might be merely due to the fact that nobody has looked for it so far. It is to be recommended, therefore, that the basal segment of the sympod of the second leg be carefully examined in all studies of the genus *Lepeophtheirus* in the future.

In common with other species of the genus, the female of *L. cuneifer* changes greatly in general appearance between the pre-adult and the adult stage, the main difference being evident in the shape of the genital complex. Its juvenile condition is represented in Fig. 4.

Acknowledgments

I wish to thank Dr E. L. Bousfield for making available to me the specimens on which description of this species has been based.

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Growth and Survival of White Suckers (*Catostomus commersoni*) in an Acidified Lake

RICHARD J. BEAMISH

Department of the Environment
Fisheries and Marine Service, Freshwater Institute, Winnipeg, Man. R3T 2N6

BEAMISH, R. J. 1974. Growth and survival of white suckers (*Catostomus commersoni*) in an acidified lake. J. Fish. Res. Board Can. 31: 49–54.

White suckers (*Catostomus commersoni*) in the acidic Lumsden Lake in 1967 and 1968 exhibited reduced annual growth followed by death. The reduced growth and death appeared directly related to the low *p*H and not to a shortage of food caused by the decreasing *p*H.

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Les meuniers noirs (*Catostomus commersoni*) d'un lac acide, le lac Lumsden, n'ont pas réussi à se reproduire en 1967 et 1968, ont subi un ralentissement de croissance annuelle et moururent. La diminution de la croissance et la mort ne proviennent pas d'un manque de nourriture causé par un abaissement du *p*H. La diminution des stocks et l'insuccès de la reproduction ont été observées chez d'autres espèces dans le lac Lumsden et dans d'autres lacs.

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IN previous reports (Beamish and Harvey 1972; Beamish 1974) it was shown that lakes in the La Cloche Mountain region of Ontario, Canada, were becoming acidified. Acid precipitation, resulting from the combination of water vapor and sulfur dioxide to form dilute concentrations of sulfuric acid in the atmosphere, was considered to have caused more than a 100-fold increase in the acid concentration of some lakes. Metal smelters in Sudbury, Ont., 65 km northeast of the study area, were considered to be the largest single source of the sulfur dioxide. As a consequence of the increase in acid, fish populations had disappeared from several of the more severely acidified waters. It was the purpose of subsequent studies to examine the methods by which the increasing acid content of the lakes was causing the extinction of fish populations.

In this report the growth, population size, and food of white suckers (*Catostomus commersoni*) from Lumsden Lake (46°01′N, 81°26′W) and from the less acidic George Lake (46°02′N, 81°24′W) were examined, to determine if the resulting loss of the white sucker population in Lumsden Lake was a direct effect of low *p*H on the fish, or an indirect effect causing the reduction of food supply.

Materials and Methods

The La Cloche Mountain area, George and Lumsden lakes, and the chemistry of these two lakes have been described previously (Beamish and Harvey 1972; Beamish 1970). Hydrogen ion was determined in the laboratory within several hours of collection. Methods for the determination of the heavy metal concentrations were similar to those described in Beamish (1974).

FOOD AND GROWTH

A survey of the food of white suckers was undertaken in 1968. The amount of each organism present in the gut of white suckers was estimated as a percentage by volume of the total gut contents. Food items were preserved in a 5% formalin solution prior to microscopic identification.

Yearly growth of white suckers was estimated by counting annual marks on sections of pectoral fin rays (Cuerrier 1951; Ovchynnyk 1965; Beamish 1973a) or by the scale method for some immature fish. Growth was also estimated using tagged fish, and growth of George Lake white suckers in Lumsden Lake was estimated by introducing 107 tagged George Lake fish into Lumsden Lake in 1968. An additional 64 George Lake white suckers were handled in a similar manner and released into George Lake as controls. The majority of the white suckers for this experiment were selected to correspond in size to the average size of white suckers in Lumsden Lake. A few were selected that corresponded to the average age of the Lumsden Lake white sucker population.

POPULATION ESTIMATES OF WHITE SUCKERS IN GEORGE AND LUMSDEN LAKES

Estimates of the number of mature white suckers in each lake were made according to Petersen (1896). The optimum number of fish needed to be tagged and re-

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covered was estimated from nomograms according to Robson and Regier (1964). Fish were captured with seines or trapnets (Beamish 1973b) for tagging, and recovered using gillnets. The tagging procedure involved anesthetizing the fish in tricaine methanesulfonate (M.S.222). Fork lengths, weights, and several scales were taken from all tagged fish. No pectoral fins were removed at the time of tagging, but all recaptured individuals had the first two to four pectoral fin rays removed for age determination.

Estimate of Standing Crops of Benthic Invertebrates

During May 21–28, 1968, 159 benthic samples from George Lake and 70 benthic samples from Lumsden Lake were taken during the daylight hours. From September 23–30 a second set of 180 samples in George Lake and 80 samples in Lumsden Lake were also taken during the daylight hours. Standing crops of benthic invertebrates were estimated in Lumsden and George lakes using a KB corer (Brinkhurst et al. 1969). Sampling sites were randomly selected according to depth and according to the variation in distribution of major food items found in a preliminary survey. All the spring samples collected in Lumsden Lake and one-half the spring samples from George Lake were sorted according to the sugar-flotation technique outlined in Anderson (1959) and Gerking (1962). After sorting these samples it was apparent that hand picking organisms from the surface of the sugar solution did not recover many of the smaller organisms, was slow, and introduced a bias according to the operator's ability to find floating individuals. Therefore, the second one-half of the George Lake spring samples and all fall samples were sorted by pouring the floating organisms through 75 μ plankton netting. All organisms retained in the netting were preserved in 3% formalin. Further details concerning the selection of sampling sites and the separation procedures are available from the author or from Beamish (1970).

Results

Growth of White Suckers in Lumsden and George Lakes

In 1966 a total of 150 m of gillnet, of mesh size suitable for the capture of white suckers, was set over a 24-hr period. The amount of gillnet set for a 24-hr period was increased to 3000 m in 1967, 1160 m in 1968, 4275 m in 1969, and reduced nettings have continued each year up to 1973. In 1966, 179 white suckers were captured; 315 white suckers were gillnetted in 1967; only 3 were gillnetted in 1968, and no Lumsden Lake white suckers were captured after 1968. Trapnets set in 1968 also captured 26 white suckers. The mean fork lengths of fish of a given age decreased in 1967 and 1968 (Fig. 1). The mean fork lengths of the 1963 and 1964 year-classes (4 and 5 year-olds) in 1968 were less than the mean fork lengths of the corresponding year-classes in 1967 (3 and 4 year-olds).

Only one of the 411 white suckers tagged in 1967 was recaptured in 1968. This fish, a 4 year-old in 1968, had grown 0.7 cm in fork length and increased 2 g in weight. The average growth increment for an individual of similar age from 1966 to 1967 was 2.9 cm and 45 g. From 1967 to 1968 this individual achieved only 21% of the increment in fork length and only 5% of the increment in weight that was achieved by the average fish of similar age in 1966–1967.

Of the 107 George Lake white suckers introduced into Lumsden Lake in May–June 1968, 23 were recovered in gillnets or trapnets in June–July and removed from the lake. An additional 12 were captured in trapnets and returned to the water in good condition. Only one of the possible remaining 84 introduced white suckers was recaptured in the extensive netting program of 1969, and none thereafter. The single introduced white sucker, recaptured for the first time after marking 1 year earlier, measured 23.5 cm fork length and weighed

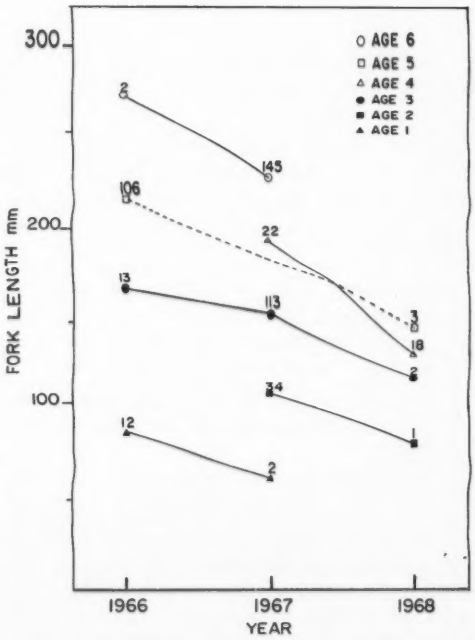

Fig. 1. Mean fork length of similar aged white suckers captured in Lumsden Lake in 1966, 1967, and 1968. No 2-year-olds were sampled in 1966. Numbers indicate size of sample in each year-class. Because of the absence of 5-year-old fish in 1967, the 1962 year-class, a broken line was used to connect the mean lengths of the 5 year-olds in 1966 and 1968.

148 g when tagged, and 23.7 cm and 109 g when recaptured. There was an estimated error of approximately 25 g in the weight of this individual as a small portion of the body cavity had been consumed by crayfish. George Lake white suckers of similar age averaged an increase of 42 mm and 300 g over the same period in George Lake.

In George Lake there was no suggestion of year-class failure, nor was there any apparent decrease in the average amount of yearly growth of fish of similar age, from 1967 to 1969 (R. J. Beamish unpublished data). While the gillnetting effort was reduced in 1968 the number of white suckers captured per unit effort was reduced only slightly from the catches in 1967. In the summers of 1971 and 1972, the *p*H of George Lake dropped below 4.8 and the average annual growth of white suckers was reduced (R. J. Beamish unpublished data).

George Lake white suckers tagged in a similar manner as those introduced into Lumsden Lake, but put back into George Lake in 1968, survived and grew at normal rates. Of these 64 George Lake white suckers used as a control for the introduction of George Lake white suckers into Lumsden Lake, five were recaptured in 1969 and two in 1970. The average annual growth of the recaptured fish in 1969 and 1970 was similar to the average annual growth of fish of similar age in 1967 and 1968. Two of the 64 control fish were recaptured in 1971 and eight in 1972, however, as growth of all white suckers in George Lake was reduced in 1971 and 1972, these fish could no longer be considered as controls. The recapture of 14 different control fish from 1969 to 1972 in the larger George Lake, with considerably less effort than applied in Lumsden Lake, indicated that tagging and handling could not be considered important in the disappearance and reduced growth of the introductions into Lumsden Lake.

Population and Biomass Estimates

An estimated 1184 (95% confidence interval of 889–1479) mature white suckers existed in Lumsden Lake in 1967. In 1967, the total catch of immature white suckers of age-class 1 and 2 represented less than 2% of the total catch by weight. Therefore, the contribution of immature white suckers in Lumsden Lake was not considered to significantly affect the calculated biomass estimate. Using the surface area to approximate the bottom area, and the mean weights of 285 white suckers for all age classes, a biomass of 0.49 g of mature white sucker per m^2 was estimated to be in Lumsden Lake in 1967. It was estimated that in 1967, George Lake contained 934 (95% confidence interval of 825–1102) mature white suckers. In George Lake immature white suckers contributed significantly to the total biomass. An estimate was made of the number of these immature individuals (under 400 mm fork length), based on the observation that between the marking period and the spring of 1969, 870 white suckers were marked or taken out of the lake that would have been in the size range for immature white suckers in 1967. Biomass values were determined for George Lake white suckers using the population estimate of 934 plus 870 individuals, and the mean weights of a sample of 600 mature and immature white suckers collected in 1967, and using the surface area of George Lake to approximate the bottom area. A value of 1.10 g of white sucker per m^2 was based on a number of immature fish known to be in the lake, and while this estimate was low because not all immature fish were identified, it was the best estimate available.

Standing Crops of Benthic Invertebrates

Cladocera and to a lesser extent chironomids were the major food of white suckers in both lakes for the period June to October. *Acantholeberis curvirostris* was the dominant food organism of white suckers during this period. Chironomids, pelecypods, and a small percentage of cladocera comprised their winter diet. The estimates of standing crops are limited to organisms found in the gut contents of the white sucker (Table 1). A complete list of organisms and the identification of major genera of chironomids can be obtained from the author. Estimates are listed separately according to the unmodified sorting method that involved hand picking organisms and the modification by pouring through 75 μ plankton netting. The 95% confidence intervals have been assigned to all estimates. Cladocera and diptera made up the greatest proportion of biomass samples, both being slightly more numerous in George Lake than in Lumsden Lake. The cladoceran *Acantholeberis curvirostris* was an abundant species in both lakes. This species was also an abundant food item of white suckers in each lake. Major species of chironomids common to the guts of white suckers in both lakes were not identified. The major genera in the benthic samples from both lakes were similar and thus it is likely that genera in the guts were similar.

Comparing the 1967 white sucker population estimates with the 1968 benthos standing crop estimates, the slower-growing white suckers in Lumsden Lake potentially had a greater amount of food organisms per unit weight than white suckers in George Lake (Table 1). The spring benthic samples indicated that white suckers had approximately 10 times the dry weight of cladocera and chironomids in Lumsden Lake than they did in George Lake. The fall standing crop determina-

tions indicated that Lumsden Lake white suckers had approximately six times the dry weight of chironomids and three times the dry weight of cladocera per unit weight of fish.

These calculations were based on 1967 population estimates of white suckers and 1968 standing crop estimates. The fishing effort indicated that the population in Lumsden Lake was greatly reduced in 1968 while the George Lake population appeared only slightly less numerous in 1968. The calculations of the amount of food per unit weight of fish are biased in favor of a reduced amount of food per unit weight of Lumsden Lake white suckers. If the true population estimates were known for 1968, the differences between the amount of food per unit weight of white sucker in each lake would be much greater.

Dry weights of some of the major groups were determined by heating at 60 C for 24 hr, using animals that were previously preserved in a 3% formalin solution. The use of dry weights determined from preserved specimens as quantitative indicators of biomass has been questioned (Johnson and Brinkhurst 1971). In this study, dry weights determined from a number of preserved specimens were used to compare approximate sizes of animals between lakes, and not with the intention of deriving absolute measurements of biomass.

Discussion

Growth

The reduced annual growth of the tagged Lumsden Lake white suckers from 1967 to 1968 and the absence of growth of the tagged introduced George Lake white sucker, while controls in George Lake grew normally, indicated that the reduced growth of the Lumsden Lake white suckers was real. Problems associated with the mechanics of determination of age or the effects of the tagging procedures could not account for the decrease in growth. Because of the intensive efforts invested, as well as the variety of gear and mesh sizes used to capture fish in Lumsden Lake throughout the study, it was unlikely that the observed growth decreases were an artifact of the sampling procedure. The decrease in mean size of the 1963 and 1964

TABLE 1. Standing crop of benthic invertebrates in George and Lumsden lakes in 1968, and the approximate weight of cladocera and chironomid larvae per gram of white sucker in each lake.

	Organism	Total number in lake/m^2	Mean dry weight of organisms[a] g×10^{-5}	Dry weight of organism per g of fish
May 21–28[b] George Lake	Total diptera	3590± 930[c]	–[d]	–
	Chironomid larvae	3270± 920	7.40(941)	0.2204
	Total cladocera	320± 90	1.30(87)	0.0030
May 21–28 George Lake	Total diptera	7000±1260	–	–
	Chironomid larvae	6140±1200	5.27(725)	0.2948
	Total cladocera	1970± 740	0.53(247)	0.0095
May 21–28[b] Lumsden Lake	Total diptera	6240±1050	–	–
	Chironomid larvae	5350± 890	20.41(543)	2.2128
	Total cladocera	650± 300	6.00(115)	0.0789
Sept. 23–30 George Lake	Total diptera	18840±3060	–	–
	Chironomid larvae	18170±3060	2.69(372)	0.4449
	Total cladocera	24160±8680	0.89(281)	0.1957
	Acantholeberis curvirostris	2350± 510	–	–
Sept. 23–30 Lumsden Lake	Total diptera	17560±2240	–	–
	Chironomid larvae	17360±2250	10.18(114)	2.5781
	Total cladocera	17280±2330	1.62(136)	0.5668
	Acantholeberis curvirostris	3240±1200	–	–

[a]Number of organisms weighed in parentheses.
[b]Unmodified sugar flotation method.
[c]95% confidence limit.
[d]Dash indicates no measurement.

year-classes from 1967 to 1968 appeared to result from a selective mortality of the larger individuals within the population as the decrease in *p*H approached lethal levels.

In general the diets of white suckers in both lakes were similar as were the major species of cladocera and genera of chironomids. As shown, there was potentially more of the major food items per unit weight available to the Lumsden Lake white suckers. A comparison of the white sucker biomass in each lake also indicated that the white suckers in Lumsden Lake were probably slow-growing (Beamish 1973a) as a consequence of environmental pressures and not as a result of overcrowding.

Disappearance of Fishes

Other fishes in Lumsden Lake appeared to have disappeared despite the presence of adequate food reserves. Before 1960, two major predators existed in the lake, the lake trout (*Salvelinus namaycush*) and the burbot (*Lota lota*). The last lake trout captured was taken in 1967 and no burbot were captured during the study. Both these species disappeared from the lake while sizeable populations of common food items (lake herring (*Coregonus artedii*), lake chub (*Couesius plumbeus*), white sucker, and crayfish) existed.

Lake trout became extremely rare in gillnet catches in George Lake by 1971 and 1972 as the *p*H decreased below 4.8 (R. J. Beamish unpublished data). Burbot, walleye (*Stizostedion vitreum vitreum*), and smallmouth bass, (*Micropterus dolomieui*) that at one time were common in George Lake, were not captured after 1970. The decrease in abundance or disappearance of these species occurred even though prey were still abundant. Lake trout also disappeared in nearby O.S.A. Lake before the disappearance of prey species (Beamish 1974).

Cause of Reduced Growth and Ultimate Mortality of Lumsden Lake White Suckers

Both lakes are deep oligotrophic lakes that are thermally stratified in the summer and have adequate oxygen concentrations (Beamish 1970). Beamish (1974) has shown that lakes in the La Cloche Mountains differ from other dilute lakes primarily in their low *p*H, and high sulfate and heavy metal concentrations.

A *p*H of 6.8 in September 1961 was the earliest recorded value for Lumsden Lake (Beamish and Harvey 1972). Determinations of *p*H in 1967 and 1968 were not reliable because of inherent *p*H changes during the period between collection and determination of samples. This problem was corrected in early 1969 and an average annual *p*H of 4.6 for Lumsden and 5.2 for George Lake was estimated for 1969. It was concluded from subsequent studies of the rate of acidification of both lakes (R. J. Beamish unpublished data) that in 1967 and 1968 Lumsden Lake probably ranged in *p*H from 5.0 to 4.5 and George Lake from 6.0 to 5.5. The higher *p*H in George Lake in 1969 and that estimated to exist in 1967 and 1968 appeared acceptable for the normal growth of white suckers.

A reduction in food utilization by chinook salmon (*Oncorhynchus tshawytscha*) in sublethal concentrations of kraft mill effluents was reported by Tokar (1968). A decrease in feeding intensity was observed for white suckers held for several months in sublethal concentrations of acid (Beamish 1972). It is suggested that white suckers in Lumsden Lake may have behaved in a similar manner and reduced feeding or food utilization or both as *p*H levels became lethal.

Other possible contaminants such as heavy metals also appear to be entering the lakes in the precipitation (Beamish 1974), and possibly through increased weathering rates. Heavy metal determinations for both lakes in 1972–73 (R. J. Beamish unpublished data) showed that of the heavy metals zinc, copper, iron, nickel, lead, and cadmium, only zinc levels (24–33 µg/liter in Lumsden Lake and 19–45 µg/liter in George Lake) were sufficiently high to be potentially hazardous to fishes (Canadian Department of Environment 1972). The observed zinc concentrations were well below incipient LC50 values considered representative for most salmonid fishes in water of a hardness similar to the study lakes (Canadian Department Environment 1972). The zinc levels were within the range of "safe" concentrations of 30 µg/liter, which had no effect, and 180 µg/liter, which caused an 83% reduction in egg production of fathead minnows (*Pimephales promelas*) in hard water (Brungs 1969). Mount (1966) reported zinc to be less toxic at lower *p*H values although his results did not agree with other published work. In a 5-year survey of trace metals in rivers and lakes of the United States of America, zinc was detected in 76.5% of the samples (Kopp and Kroner 1969). The mean concentration of 64 µg/liter in the 1207 positive occurrences was higher than the zinc concentrations in the study lakes. Fourteen day-old George Lake lake herring fry survived in George Lake water of *p*H 5.3, but 50% died within 48 hr after the *p*H was reduced to 4.9 (R. J. Beamish unpublished data). Time to 50% mortality was unchanged when fry were subjected to a solution of *p*H 4.9 consisting of one part George Lake water and three parts distilled water. Thus the concentrations of heavy metals were

reduced by 75% but survival time was unaffected. Concentrations of zinc, copper (George Lake 3 µg/liter, Lumsden Lake 1 µg/liter) and nickel (George Lake 10 µg/liter, Lumsden Lake 8 µg/liter) were below the concentrations considered "safe" for some aquatic organisms (Biesinger and Christensen 1972). Thus while zinc concentrations in the study lakes were high and the acidic nature of the water probably resulted in a greater proportion of metal in the ionic form, it was considered that they were not of sufficient magnitude to be considered the principle stressing agent. It is possible that synergistic effects of acid and heavy metals, especially zinc or acid, and the additive effect of heavy metals may have had an influence on the concentration of acid that first stressed the population. However, acid levels were in the range that have been considered unacceptable for fishes (EIFAC 1968; Mount 1974) and the low *p*H must be considered to be the principle factor affecting the growth and survival of fishes in Lumsden and other lakes.

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Phenotypic Variation in the Lake Whitefish, *Coregonus clupeaformis*, Induced by Introduction into a New Environment[1]

J. S. Loch[2]

Department of Zoology, University of Manitoba, Winnipeg, Man.

Loch, J. S. 1974. Phenotypic variation in the lake whitefish, *Coregonus clupeaformis*, induced by introduction into a new environment. J. Fish. Res. Board Can. 31: 55–62.

Adult lake whitefish (*Coregonus clupeaformis*) from Clearwater Lake and second generation adults of offspring from Clearwater whitefish transplanted to Lyons Lake were compared with respect to morphometric and meristic characters and isozymes of L-glycerol-3-phosphate dehydrogenase (GPDH). Feeding habits and abundance of pelagic and benthic foods were compared in the two lakes.

Gill raker number, lateral line scale count, and interorbital width remained constant between parental and offspring populations. Gill raker length was the main character found to differ between the populations. This was found to be related to the percentage and type of benthic food eaten. Abrasion of the gill rakers is offered as an explanation for the differences in gill raker length. Differences were found in various other meristic and morphological characters, as well as in electrophoretic phenotype frequencies of isozymes of GPDH.

Loch, J. S. 1974. Phenotypic variation in the lake whitefish, *Coregonus clupeaformis*, induced by introduction into a new environment. J. Fish. Res. Board Can. 31: 55–62.

L'auteur compare des grands corégones adultes (*Coregonus clupeaformis*) du lac Clearwater avec des adultes de deuxième génération issus de poissons provenant du même lac, mais transplantés dans le lac Lyons. Il compare les caractères morphométriques et numériques des deux groupes, ainsi que les isozymes de la L-glycérol-3-phosphate déshydrogénase (GPDH). Il met également en parallèle les habitudes alimentaires et l'abondance des organismes pélagiques et benthiques de fourrage dans les deux lacs.

Le nombre de branchicténies et d'écailles à la ligne latérale, de même que la largeur interorbitaire sont les mêmes chez la population mère que chez les descendants. La longueur des branchicténies est le caractère principal qui diffère entre les populations. Ce caractère est lié au pourcentage et au type d'organismes benthiques consommés. L'abrasion des branchicténies peut expliquer ces différences de longueur. Divers autres caractères numériques et morphologiques, de même que la fréquence des phénotypes électrophorétiques montrent également des différences.

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The whitefish, family Coregonidae, are infamous for their taxonomic problems due to the immense variation shown within various member 'species,' especially the "*Coregonus clupeaformis* complex" and the "*Coregonus artedii* complex" (McPhail and Lindsey 1970).

A recent transplantation of the lake whitefish, *Coregonus clupeaformis*, from Clearwater Lake, Manitoba, to Lyons Lake, Man. has provided an opportunity to study the phenotypic changes that can be induced by the environment. This plasticity is investigated through a comparison of various morphological characters, and one biochemical trait of the parental population, which inhabits a rather large northern Manitoba lake, and of the second generation progeny of fish, which had been transplanted into a small more southerly Manitoba lake. These comparisons are intended to locate conservative traits useful in defining the species.

In 1970, whitefish were sampled from Lyons Lake on four dates between May 21 and November

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science from the University of Manitoba, Winnipeg, Man.

[2]Present address: Department of the Environment, Freshwater Institute, 501 University Crescent Winnipeg, Man. R3T 2N6.

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3, and from Clearwater Lake on three dates between June 14 and October 24.

Description of Study Areas

Clearwater Lake (lat 54° 05′N, long 101°W) lies in a limestone region just beyond the edge of the Precambrian Shield and is part of the Saskatchewan River drainage system. Lyons Lake (lat 49° 46′N, long 95°W) lies just within the Precambrian Shield; it is a headwater lake, receiving no permanent inflow of water, and drains northerly into Hunt Lake, which is tributary to West Hawk Lake. The entire area is part of the larger Winnipeg River drainage system.

In 1960, after poisoning of Lyons and Hunt lakes in 1959, 7000 whitefish fry from Clearwater whitefish eggs, overwintered in the Whiteshell Trout Hatchery, were planted in Lyons Lake. A fine steel grate was placed in the outflow of Hunt Lake in 1959 to prevent entry of fish from West Hawk Lake (which has a natural whitefish population). Two yearling whitefish which were taken in Hunt Lake in 1961 are considered to be strays from Lyons, as yearling whitefish of similar size were taken in Lyons at the same time. There are no other records of whitefish in Hunt Lake; therefore, whitefish from Lyons Lake are apparently all of Clearwater Lake whitefish ancestry.

As most of the physico-chemical traits of the two lakes differed (Table 1), Lyons and Clearwater appear to provide a wide contrast in environments. Perhaps the most salient feature is that the temperature–oxygen conditions combined to leave only about 5 m of water column actually available to whitefish in Lyons Lake. Lyons Lake suffers from depletion of oxygen in the lower strata (below 10 m) during the summer. No such oxygen depletion exists in Clearwater Lake.

Methods and Materials

All whitefish were taken by gillnets, except the last Clearwater collection which was from a poundnet. All gillnets were set on the bottom at the following depths: Lyons, 5–10 m; Clearwater, 10–20 m. A total of 258 fish (fork lengths 26–43 cm) were examined from Lyons, and 281 fish (30–53 cm) from Clearwater. Fork length, weight, and sex of the whitefish were recorded at the time of capture. Fish were preserved in 10% formalin and later transferred to 40% isopropyl alcohol.

The following measurements were made on preserved specimens from the July and August samples: caudal peduncle depth (CPDEP), dorsal fin length (DORSL), anal fin length (ANALL), pectoral fin length (PECTL), ventral fin length (VENTL), adipose fin height (ADIPH), head length (HEADL), snout length (SNLEN), postorbital (POSTO), upper jaw (UPJAW), and occipital width (OCCWD) after Hubbs and Lagler (1964); snout to occipital distance (SNOCC), dorsal to adipose distance (DLADP), adipose to caudal distance (ADPCL), pectoral to ventral distance (PECVL), and ventral to anal distance (VLANL) were made after Koelz (1929); the measurements for fork length (FORKL) and pectoral origin (SNPEC) were made following Lindsey (1963); interorbital width (IORWD) and occipital depth (OCC DP) were taken from Lindsey (1962) and interorbital depth (IORDP) and internaral width (NARWD) after Svardson (1950). Other measurements were occipital to dorsal distance (OCCDL), the shortest distance between the back of the occiput to the origin of the dorsal fin; anal to caudal distance (ANLCL), the shortest distance from the origin of the anal fin to the fork of the caudal fin; predorsal depth (PREDD), the depth through the origin of the dorsal fin; postdorsal depth (POSTD), the depth through the back of the dorsal fin base; anal depth (ANLDP), the depth through the origin of the

TABLE 1. Physico-chemical features of Lyons Lake and Clearwater Lake, Manitoba, in late July, 1970.

		Lyons Lake		Clearwater Lake
Location	Coordinates	Lat 49°46′N, Long 95°W		Lat 54°05′N, Long 101°W
Area	Total	0.3 km²		295 km²
% by stratum	0–5 m	15	0–10 m	42
	6–10 m	35	11–20 m	35
	11+ m	50	21+ m	23
Depth	maximum	18 m		36 m
	mean	3 m		14 m
Transparency	(Secchi disc)	3 m		7 m
Oxygen	surface	10 ppm		10 ppm
	bottom	1 ppm		10 ppm
Temperature	surface	25.6 C		17.8 C
	bottom	4.4 C		9.0 C
Location of thermocline		4 m		15 m
Total dissolved solids		125 ppm		189 ppm

anal fin; body width (BODWD), the width through the origin of the dorsal fin; eye diameter (EYEDI), the shortest distance between the orbits as taken through the center of the eye in a horizontal plane; gill raker length (GRLEN), the shortest distance, using pointed calipers and under magnification, from the tip to the base of the gill raker located in the angle of the first left gill arch. (See fig. 2 in Lindsey 1962).

All measurements were done on the left side of the fish using dial calipers reading to 0.1 mm. These measurements were transformed to natural logarithms and plotted as Y variables against an X variable following the standard growth analysis procedure (Martin 1949). Regressions of the following measurements were calculated on the X variable, head length: SNLEN, EYEDI, POSTO, UPJAW, OCCWD, IORWD, NARWD, OCCDP, IORDP; regressions of all other measurements were made on the X variable, fork length. The resultant relationships for the two populations were compared, using analysis of covariance and a graphical interpretive method using confidence intervals for the mean Y for any X. Both statistical methods are found in Snedecor and Cochran (1967). For all measurements showing a significant Bartlett's test for homogeneity of variance, the variances were calculated and compared; the population with the larger variance was noted.

Meristic counts were made with the naked eye except for gill raker number which was counted under a dissecting microscope. The following counts were made after Lindsey (1962): gill raker number (GR), lateral line scales (LLS), caudal peduncle scales above the lateral line (CPA) and below the lateral line (CPB), dorsal fin rays (DR), anal fin rays (AR), ventral fin rays (VR), and pectoral fin rays (PR).

The counts for the two populations were compared using either a '*t*' test or Mann Whitney U Test depending upon the particular distribution to which the meristic count conformed.

The possibility of sexual dimorphism was considered for all measurements.

In addition, body contour (CT) was measured; this was the angle subtended by the origin of the dorsal fin and the back of the occiput, using a horizontal through the back of the occiput as the base line. This measurement attempted to quantify the "humpbackness" of a whitefish with one measurement. As the measurements were in whole numbers (degrees) and found to be linearly independent of fork length, they were compared using the same statistical methods used for meristic counts.

As support for the analysis of covariance, factor analysis (Cattell 1965a, b) was done using as variables — body proportions. meristic traits, body contour, fork length, and weight. Factor analyses using "sex" as an additional variable were done for each lake and then both lakes together; in the latter analysis, "location" was used as an extra variable as well as "sex." "Males" and "Lyons Lake" were coded with the integer "1," and "females" and "Clearwater Lake" as the integer "2."

Isozymes of L-glycerol-3-phosphate dehydrogenase (GPDH) were analyzed using electrophoretic procedures described in Lindsey et al. (1970). Whitefish from the October sample (Clearwater Lake) and the November sample (Lyons Lake) were used. The isozymes of lactate dehydrogenase (LDH) and hemoglobin have been previously analyzed by electrophoresis for the two populations and results are mentioned in this paper.

To attempt to relate possible phenotypic differences observed between the two populations with environmental causes, the food availability and whitefish diet in the two lakes was investigated.

Food availability was determined by plankton and benthic fauna sampling. Plankton was sampled with a no. 10 Wisconsin net at the deepest known point in each lake. One total vertical tow was taken at each sampling time. Bottom fauna was sampled using a 15 cm Ekman dredge. All macroscopic organisms were removed and preserved in 40% isopropyl alcohol for identification and quantitative study. The benthic sampling scheme involved a random stratified sampling method: the lakes were divided into strata (Lyons 0–5 m, 6–10 m, 11+m; Clearwater: 0–10 m, 11–20 m, 21+m), and four stations were chosen at random within each stratum. The number of stations per stratum was determined by considering the percentage area of the lake that stratum occupied, and the density of benthic fauna in each stratum, as determined by a preliminary survey. The overall estimate of the population of each organism was determined after Snedecor and Cochran (1967). Weights of benthic and pelagic forms were taken, each sample being blotted dry before weighing to remove excess fluid.

Diet of the fish was investigated by stomach analysis. The entire stomach was removed by severing the esophagus and pylorus and then was placed in 10% formalin. Stomach contents were divided into two general categories: benthic (those organisms that are almost exclusively benthic), and pelagic (those organisms that are partially or totally pelagic) (Kliewer 1970). That portion of the pelagic category which comprised pelagic cladocerans and copepods was recognized as a third category, crustacean. These were felt to be surer indicators of pelagic feeding. Percent settled volume was used as a measure of quantity. Only the May 21 and July 21 (Lyons) and June 14 and August 4–5 (Clearwater) samples were analyzed for stomach contents.

In order to relate morphological changes to possible growth rate differences, all fish used in morphological comparisons were aged as well. Age determinations from scales were made by the author following standard techniques (Lagler 1956). Scales for age determination were removed from the upper dorsolateral surface of the fish. Regressions of fork length on scale radius were calculated for Lyons and Clearwater regardless of sex using the Lee method (Lagler 1956). The expected fork lengths for the 1965 year class were calculated for the average annuli. These fork lengths were plotted against age for the two lakes as an approximate indicator of differences in growth rate between the two populations for the same aged fish.

Results

Whitefish were aged to 6 years in Lyons Lake and at least 10 in Clearwater. Since 6 years appears

to be the maximum lifespan of whitefish in Lyons Lake, yearling whitefish which were captured there in 1970 can be regarded as evidence that a self-reproducing population of at least three generations has been established in Lyons Lake. The growth curves for the two 1965 year-classes (Fig. 1) show that the Lyons Lake whitefish were larger at all ages than the same year-class in Clearwater Lake.

Results of the relative growth comparisons, using graphical interpretation from the confidence bands for the mean Y for any X, can be subdivided into three categories, one example of each being shown in Fig. 2: (1) those body proportions that showed overlap of 95% confidence intervals over the entire length interval ("full overlap"), that is, conservative traits — BODWD, POSTD, PECTL, VLANL, DLADP, NARWD, PREDD (males), ANLDP (females); (2) those body proportions that showed overlap at some lengths, but not at others ("partial overlap") — ANALL, VENTL, SNPEC, ADPCL, HEADL, OCCDL, ANLCL, PECVL, CPDEP, ADIPL, EYEDI, IORWD, UPJAW, SNLEN, OCCDP, OCCWD, POSTO, GRLEN, weight, PREDD (females), SNOCC (females); (3) those proportions that showed no overlap over the entire length interval ("no overlap"), that is, very plastic traits — IORDP and SNOCC (males) which were larger in Clearwater whitefish and ANLDP (males) and DORSL (males) which were larger in Lyons whitefish. Analysis of covariance of the relative growth lines gave similar results. All rayed fins were significantly longer for Lyons whitefish with the exception of pectoral length (which showed no difference between populations). Sexual dimorphism was indicated in DORSL, ANALL, VENTL, PECTL, PREDD, POSTD, ANLDP, SNOCC, and POSTO (all of which were significantly larger in the females). However, traits that were sexually dimorphic in Lyons (POSTD, ANLDP, POSTO, weight) were not the same as those for Clearwater (SNOCC, DORSL, ANALL, PECTL, VENTL) except for PREDD.

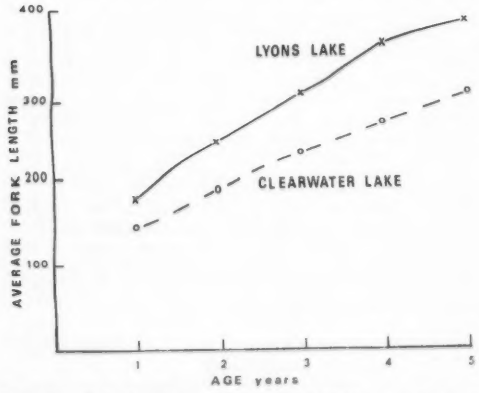

FIG. 1. Calculated growth rates of 1965 year-class of whitefish from Lyons Lake and Clearwater Lake.

Factor analysis showed that the major portion of the variation found in VENTL, DORSL, ANALL, ADIPH, GRLEN, OCCDP, and IORDP could be related to the specific population from which the measurement was taken. Therefore, these are the traits most readily susceptible to environmental pressure. Results using this statistical method are comparable with the results from the other statistical methods used.

Among the meristic traits, significant differences were found between the populations for caudal peduncle scale count above and below the lateral line and dorsal and anal fin ray numbers. No differences were found for gill raker number, lateral line scale count, and pectoral and ventral fin ray numbers. Body contour was significantly different between populations (Table 2).

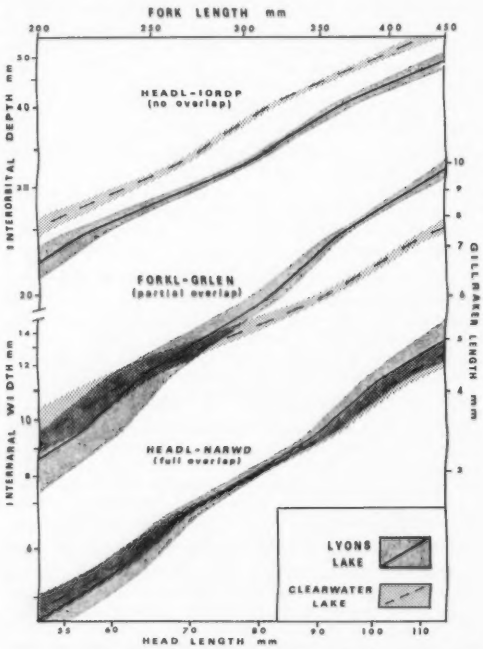

FIG. 2. Fitted regression lines and 95% confidence intervals for interorbital depth (IORDP) and internaral width (NARWD) on head length (HEADL), and gill raker length (GRLEN) on fork length (FORKL) for whitefish populations from Lyons Lake and Clearwater Lake.

Frequencies of electrophoretic phenotypes for the isozymes of L-glycerol-3-phosphate dehydrogenase were found to be significantly different between Lyons and Clearwater whitefish (Table 3).

Complete numeric results for all comparisons mentioned above are found in Loch (1971).

Discussion

Relative Growth Analysis

Relative growth plots for fishes are characterized by a series of stanzas, each with a different relative growth constant. The transition from one stanza to the next generally takes place by means of a sharp growth inflection (Martin 1949). Martin found that environmentally induced differences in body size at growth inflection and homogeneous (parallel) slopes indicate little variation in the subsequent rate of growth. Experimental work showed that either temperature or diet could produce differences in size at inflection.

In this study several body proportions appear to have highly significant differences in slopes of the relative growth plot ($P<0.01$). Either these measurements are dependent on growth rate after juvenile growth inflections and, therefore, are susceptible to the environment, or differing slopes are determined at an early age. Of particular interest are those measurements that show diverging slopes (gill raker length and occipital depth); here the differences between populations increase with length of the fish. Martin (1949) induced the same result for the length–weight relationship using a deficient diet (causing a slow growth rate). Alternatively, these divergences could have been determined at an early age and be independent of subsequent growth rate.

Heterogeneity of Gene Pool

The heterogeneity of variance may have biological meaning in this study: increased variance in one population might represent actual genetic heterogeneity inherent in this population. Comparison of variances where they differed significantly showed that Clearwater Lake in all 14 cases had a higher error variance associated with it. This could indicate that the gene pool in Clearwater Lake is more heterogeneous than in Lyons Lake, due to existence of subpopulations in Clearwater. The increased heterogeneity could also be due to the Founder Principle — the transplants in Lyons Lake had a smaller gene pool available than that of the total Clearwater population. Alternatively, the Clearwater whitefish may have the same gene pool

TABLE 2. Means and ranges of meristic traits of Lyons Lake and Clearwater Lake whitefish.[a]

	GR	LLS	CPA[b]	CPB[b]	DR[b]	AR[b]	PR	VR	CT[b]
Lyons Lake	26.9 (25–29)	82.7 (75–89)	11.6 (10–13)	11.3 (10–12)	10.8 (11–13)	11.4 (10–13)	15.9 (15–18)	11.1 (10–12)	17.5° (9–27)
Clearwater Lake	25.9 (25–30)	82.5 (77–87)	11.3 (10–12)	10.8 (10–12)	11.0 (11–13)	10.9 (11–13)	15.7 (14–17)	11.3 (11–12)	12.9° (1–27)

[a]Sample size was 63 whitefish from each lake.
[b]Measurement showed significant difference ($P<0.05$) between means using 't' test.

TABLE 3. Comparison of gene and phenotype frequencies of L-glycerol-3-phosphate dehydrogenase from whitefish in Lyons and Clearwater lakes.

	Calculated gene frequency[a]			Observed phenotype frequency[b]					
	b_1	b_2	b_3	B_1B_1	B_2B_2	B_3B_3	B_1B_2	B_1B_3	B_2B_3
Lyons Lake	0.470	0.220	0.309	19	2	6	30	43	18
Clearwater Lake	0.411	0.191	0.398	24	5	20	16	37	21

[a]Comparison of gene frequencies of two lakes gives $\chi^2 = 4.15$, 2df, $P = 0.1$
[b]Comparison of phenotype frequencies of two lakes gives $\chi^2 = 14.18$, 5df, $0.025>P>0.01$

available for variation as do Lyons whitefish, but increased diversity in habitat in Clearwater has produced increased phenotypic plasticity.

Growth Rate and Body Form

It seems that there is no necessary correlation between overall body form and growth rate. In this study, Lyons Lake fish with the faster growth rate did have smaller heads, but their fins were larger. Hart (1931) found that fast-growing whitefish from Lake Ontario had smaller heads and fins compared with slower-growing fish from Lake Nipigon. Martin (1949) believed this to be the general correlation expected.

Another point of interest regarding body form is the difference in "humpbackness." The fact that the low profile of a Clearwater whitefish could be altered so significantly within two generations in Lyons Lake indicates an environmental influence on "humpbackness."

Gill Raker Length and Diet

An environmental explanation can be suggested for the differences in gill raker length: they are due simply to physical abrasion. Perhaps fish that feed on benthic animals such as snails, which are harder than zooplankters, have shorter gill rakers due to mechanical wear on the gill raker during feeding. The Clearwater Lake whitefish diet is comprised of snails, clams, and amphipods, all certainly more abrasive than the Chironomidae, *Chaoborus*, and zooplankton which make up the Lyons Lake whitefish diet (Table 4 and 5). The diverging regression lines for gill raker length (Fig. 2) could also be explained by this abrasion theory: the older the fish the more wear on the gill rakers. In addition, in both Lyons and Clearwater lakes, when the stomach contained benthic organisms, a considerable amount of substrate was found in the stomach as well. In Lyons Lake the substrate was mud, while in Clearwater Lake the substrate was sand, certainly more abrasive than mud.

If the major food available is pelagic, the feeding mechanism may require long gill rakers to serve as a sieving device. At the same time, lack of abrasion may permit the gill rakers to remain long. If the major food source is benthic, a sieving device may still be needed if the whitefish acts as a vacuum, in which case the substrate and food organisms may act as an abrasive on the gill rakers. Therefore, it is possible that the gill raker length is determined not solely by natural selection but perhaps also by the relative ability of the food organisms and substrate ingested to cause wear on the gill rakers.

Kliewer (1970) found a strong negative correlation between gill raker length and the percentage of benthic food eaten. The conventional explanation for this is that longer gill rakers enhance the sieving capabilities of the gill apparatus and that this is an adaptation for pelagic feeding.

This study shows similar results: Clearwater Lake whitefish have much shorter gill rakers than Lyons Lake whitefish and feed almost exclusively on benthic organisms. However, in the artificial classification of pelagic as defined by Kliewer, there are certain organisms which are 'benthic' at some times and 'pelagic' at others, such as *Chaoborus* which follows a diurnal pattern, migrating to the surface at night and remaining on the bottom in deep water in the day. In addition, some species of cladocerans may also be transiently benthic. Since *Chaoborus* occurred in 60% of Lyons Lake sto-

Table 4. Food availability and diet of whitefish in Lyons Lake and Clearwater Lake.

	Lyons Lake						Clearwater Lake					
	May			July			June			August		
Food type[a]	B	P	C	B	P	C	B	P	C	B	P	C
Diet												
stomach samples (% volume)	60	27	0	5	95	75	92	6	0	99	0	0
Food abundance												
Bottom fauna[b]		0.037			0.034			0.088			0.105	
Plankton[c]		0.1			0.2			0.1			0.1	

[a]Food type — B: Benthic; P: Pelagic (total); C: Crustacean (pelagic Cladocerans and Copepods).
[b]Weight in g/sample.
[c]ml settled volume/m of water column.

TABLE 5. Composition of stomach and bottom samples of Lyons and Clearwater lakes.

	Lyons Lake				Clearwater Lake			
	May		July		June		August	
	SS[a]	BS[b]	SS	BS	SS	BS	SS	BS
Diptera (*Chaoborus* larvae)	60	33	60	10	0	0	0	0
(Chironomidae larvae)	90	47	20	73	45	22	44	15
(Dipteran pupae)	10	0	35	0	0	0	0	0
(Dipteran adults)	15	0	10	0	0	0	0	0
Hemiptera	35	0	0	0	0	0	0	0
Ephemeroptera	5	0	0	0	15	0	0	0
Gastropoda	15	1	5	3	75	25	19	0
Pelecypoda	0	0	0	1	75	0	63	0
Amphipoda	5	2	0	1	55	46	81	81
Trichoptera	0	0	0	0	50	3	6	0
Odonata	0	18	0	1	0	0	0	0
Hirudinea	0	0	0	0	25	0	0	0
Oligochaeta	0	1	0	13	0	0	0	0
Fish	0	0	5	0	0	0	0	0
Plants	5	0	10	0	25	0	0	0
Crustacea (Cladocera and Copepoda)	0	0	75	0	0	0	0	0

[a]Stomach samples — percent occurrence of various groups in a total of 20 samples per month.
[b]Benthic samples — percent occurrence of various groups in a total of 12 samples per month.

machs in both May and July, the 'pelagic' component may have been overestimated in this study. Lyons Lake fish contained 75% crustacean plankton in July, while none appeared at any time in any Clearwater Lake fish (Table 4).

Kliewer (1970) felt that the relationship between raker length and diet had arisen gradually through natural selection. While this study shows the same relationship between raker length and diet and also a contrast in food availability between lakes, time allowed for natural selection (two generations) appears to be inadequate for such a large divergence in gill raker length (Fig. 2) between the two populations. The Founder Principle could have operated here due to the relatively small number of fish used in the transplant and the chance of variant genes having expressed themselves. However, the probability of such genes being present in Lyons stock is small, due to the random fertilization of whitefish at Clearwater Lake and the random method of selection of whitefish stock from the hatchery. Therefore, it is felt that environmental factors rather than genetic ones most likely caused the difference in gill raker length.

IMPLICATIONS OF THE BIOCHEMICAL ANALYSIS

The electrophoretic phenotype frequencies of the isozymes of L-glycerol-3-phosphate dehydrogenase (GPDH) appear to have changed between populations (Table 3). Franzin and Clayton (1971) found that various crosses of whitefish produced GPDH electrophoretic phenotype ratios predictable by simple Mendelian inheritance and, therefore, one would not expect to find the difference observed in this study. Comparison of the gene frequencies shows that the numbers of genes in the two lakes are in fair agreement. Therefore one might conclude that there is some environmental influence on the GPDH electrophoretic phenotypes. However, a further examination was made of the numbers of phenotypes within the lakes by comparison of the observed numbers of phenotypes to the numbers expected based on the usual Hardy-Weinberg calculation from the gene frequencies.

	Phenotype number					
Lyons Lake	B_1B_1	B_2B_2	B_3B_3	B_1B_2	B_1B_3	B_2B_3
obs	19	2	6	30	43	18
exp	26.1	5.7	11.3	24.5	34.5	16.1
	$x^2 = 10.51$, 5 df, $0.1 > P > 0.05$					
Clearwater Lake						
obs	24	5	20	16	37	21
exp	20.7	4.5	19.5	19.3	40.2	18.7
	$x^2 = 1.68$, 5 df, $0.9 > P > 0.8$					

No significant differences were found within the two populations at $P < 0.05$.

Therefore, there is indication that GPDH electrophoretic patterns may be influenced by nongenetic causes.

Lindsey et al. (1970) compared Lyons Lake and Clearwater Lake whitefish for hemoglobin types and the ratios of the types were consistent between the lakes. Similar results were found for lactate dehydrogenase (J. W. Clayton personal communication). Hence, these biochemical traits were not affected by the transplant of whitefish.

The morphometric differences observed in this study could be due either to genetic differences, or environmental effects, or a combination of the two. The fact that the populations differed with respect to the GPDH phenotypes does not imply that morphological differences observed were due to genetic differences. The hemoglobin patterns provide direct evidence of a trait in which no genetic differences appear to exist between the populations. Furthermore, the best explanation of the differences in gill raker length appears to be based on phenotypic causes.

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Composition minérale des écailles du *Catostomus commersoni* issu de deux milieux différents: étude par microscopie électronique analytique[1]

R. VAN COILLIE ET A. ROUSSEAU

Institut national de la recherche scientifique Université du Québec, Ste Foy, Qué.
Avec la collaboration de G. VAN COILLIE
Secrétariat général de l'Université du Québec

VAN COILLIE, R., ET A. ROUSSEAU. 1974. Composition minérale des écailles du *Catostomus commersoni* issu de deux milieux différents: étude par microscopie électronique analytique. J. Fish. Res. Board Can. 31: 63–66.

La composition minérale des écailles, limitée aux métaux, a été étudiée chez le *Catostomus commersoni* vivant dans un milieu sain (rivière Manicouagan) et chez la même espèce vivant dans un milieu pollué entre autres par divers métaux (rivière des Prairies près de Montréal); à cette fin, on employa la microspectrométrie aux rayons X combinée à la microscopie électronique par balayage et un balayage microdensitométrique subséquent. Les écailles contiennent, en plus du Ca largement prédominant, du Na, Mg, Al, K, Fe, Cu, Zn et Ba; on y détecte aussi un peu de Cr, Mn, Co, Ni, Sr, Ag, Cd, Cs, Hg et Pb. Tous ces métaux sont surtout situés entre les circuli dans ces structures ostéoïdes. Les métaux précités dans la seconde énumération ci-dessus, généralement reconnus pour leur toxicité, sont plus abondants dans les écailles du catostome issu du milieu pollué que dans celles du catostome provenant du milieu sain; c'est la situation contraire pour les autres métaux analysés, excepté pour l'Al et le Ba en quantités sensiblement égales dans les deux séries d'écailles. Si l'on met en parallèle ces différences dans la composition minérale des écailles du catostome des deux milieux concernés, le fait que les métaux toxiques précités soient plus abondants dans le milieu pollué et la possibilité qu'un métal en excès dans un milieu soit incorporé dans les écailles à la place du calcium, on peut penser que la composition minérale des écailles est en relation, au moins partielle, avec celle de l'environnement aquatique.

VAN COILLIE, R., ET A. ROUSSEAU. 1974. Composition minérale des écailles du *Catostomus commersoni* issu de deux milieux différents: étude par microscopie électronique analytique. J. Fish. Res. Board Can. 31: 63–66.

The mineral content of scales, metals only, was studied. Fish of the *Catostomus commersoni* subspecies were used, taken from a healthy environment, (the Manicouagan River) and an environment polluted by, among other substances, various metals (Rivière des Prairies, near Montréal). X-ray microspectrometry was combined with electron microprobe scanning techniques, with subsequent microdensitometric scanning. Scales contained, in addition to the highly predominant calcium, the following: Na, Mg, Al, K, Fe, Cu, Zn, and Ba, as well as some Cr, Mn, Co, Ni, Sr, Ag, Cd, Cs, Hg, and Pb. All metals were between the circuli in the osteoid structures. The latter category of metals, widely known for their toxicity, were more abundant in the scales of the sample from polluted waters, whereas the reverse was true for the other metals, with the exception of Al and Ba, which were found in equal quantities in both samples. If the difference in content of the scales taken from fish of the two different environments is seen in relation with the fact that the toxic metals are more abundant in polluted waters and the fact that there is the possibility that excess metal from the water can replace calcium in the scales, we may infer that the mineral content of scales is related at least in part to that of the surrounding aquatic environment.

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CHEZ le poisson, comme chez tout pluricellulaire, le milieu intracellulaire a une composition chimique qui fluctue généralement assez peu tandis que celle du milieu extracellulaire interstitiel peut varier beaucoup plus et dépendre, partiellement au moins, de l'adaptation écologique de l'individu à son milieu de vie. Dans cette optique, nous avons voulu vérifier si la composition minérale des écailles varie chez la même espèce vivant dans deux milieux différents et, à cette fin, nous avons employé une nouvelle approche expérimentale basée sur la microscopie électronique analytique.

Matériel et Méthodes

Les écailles furent retirées de la région située entre la nageoire dorsale et la ligne latérale chez le catostome noir, *Catostomus commersoni*, pris en juillet–août 1972 dans la rivière Manicouagan (latitude: environ 50°39′ et longitude: environ 68°45′), sur la Côte-Nord du golfe du Saint-Laurent, ou dans la rivière des Prairies (latitude: 45°39′ et longitude: 73°33′) à la périphérie de Montréal. Après les avoir débarrassées des tissus voisins et après avoir vérifié qu'elles n'étaient pas des écailles de régénération, les écailles furent préparées pour la microscopie électronique à balayage effectuée avec l'appareil JEOL JXA-50A. Les détections microspectrométriques des rayons X émis par les éléments présents dans l'écaille soumise au faisceau électronique du microscope furent réalisées avec la microsonde à cristaux adjointe au microscope pour les champs postérieur, antérieur et latéraux de chaque écaille en veillant à utiliser pour chaque élément considéré la même intensité du faisceau électronique et les mêmes conditions de balayage microscopique. Lors de ces balayages microspectrométriques, des microphotographies furent ensuite prises selon les mêmes paramètres photographiques pour visualiser les différents métaux présents dans la même région d'une écaille; leurs négatifs furent ultérieurement analysés par balayage microdensitométrique avec l'appareil Joyce Loebl 3C, ce qui fournit une valeur pour chaque métal analysé. On décomptait ensuite de chaque valeur ainsi acquise le bruit de fond déterminé de la même façon à la périphérie de l'écaille. Dans tous les cas, les résultats obtenus pour un métal donné différaient peu entre les quatre champs d'écaille examinés, entre la dizaine d'écailles retirées du même individu et entre les poissons issus du même milieu de telle sorte qu'on put en tirer des moyennes dont la signification statistique indiquait une homogénéité d'échantillonnage à 95% de probabilité.

Résultats

La microscopie électronique analytique à balayage précise non seulement le relief de la face supérieure des écailles où l'on observe une disposition concentrique de crêtes (Fig. 1), appelées "circuli" (van Oosten 1957: p. 226), mais aussi la localisation des métaux dans ce relief: ces derniers sont surtout situés dans les dépressions entre les circuli comme l'indique l'emplacement des points imprimés sur les microphotographies réalisées lors de l'analyse microspectrométrique pour chaque élément (Fig. 2a et 2b). On remarque alors aussi que le nombre de ces points imprimés par unité de surface varie avec les éléments analysés dans les mêmes conditions microscopiques et photographiques (comparer Fig. 2a et 2b). Cette densité de points, mesurée par balayage microdensitométrique, est notamment beaucoup plus élevée après un balayage microspectrométrique ajusté pour le calcium qu'après tout balayage analogue réglé pour les autres métaux présents dans les écailles; dans ces conditions, la valeur de la densité micrographique occasionnée par le calcium peut servir à établir des rapports de densité micrographique entre un métal et le calcium. Ces rapports (Tableau 1) apparaissent plus élevés pour les écailles du catostome de la rivière des Prairies que pour celles du catostome de la rivière Manicouagan en ce qui concerne les métaux suivants: Sr, Mo, Mn, Cs, Cd, Ag, Hg, Cr, Co, Pb et Ni. On constate, par ailleurs, le contraire pour les autres métaux analysés mis à part l'Al et le Ba pour lesquels lesdits rapports sont presque semblables dans les deux séries d'écailles.

Discussion

Etude des caractéristiques minérales des écailles à l'aide de la microscopie électronique analytique: pour détecter les éléments présents dans un tissu biologique, la microscopie analytique par transmission et/ou par balayage commence à être utilisée (Tousimis 1969; Chandler et Chou 1970; Hall 1971; Henderson 1972). On sait que la spectrophotométrie à absorption atomique nécessite à cette fin beaucoup de matériel biologique, demande plusieurs préparations chimiques différentes pour celui-ci et doit tenir compte de certains masquages entre les éléments. Par contre, l'analyse avec un spectromètre à cristaux combiné à un microscope électronique requiert peu de matériel biologique, n'exige pour celui-ci qu'une seule préparation exempte de difficultés particulières, s'effectue rapidement sur le même spécimen pour les différents éléments, permet d'éviter les erreurs liées aux interférences d'absorption et précise la répartition des éléments dans les structures tissulaires et cellulaires. Même si les résultats fournis par cette méthode ne peuvent exprimer des quantités absolues à moins de faire appel à des standards de comparaison qui font l'objet de recherches actuelles (Lehrer et Berkley 1972), ils constituent toutefois des données qui, en plus d'apporter des indications qualitatives, s'avèrent comparables pour le même élément dans les mêmes conditions microspectrométriques entre des spécimens différents.

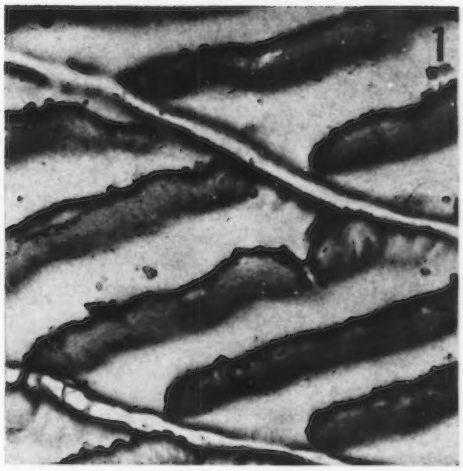

FIG. 1. Microphotographie obtenue par la microscopie électronique à balayage pour le champ antérieur d'une écaille de *Catostomus commersoni*. On distingue nettement sur cette partie d'écaille des crêtes parallèles dénommées "circuli" et, entre celles-ci, des dépressions. G = 300 ×.

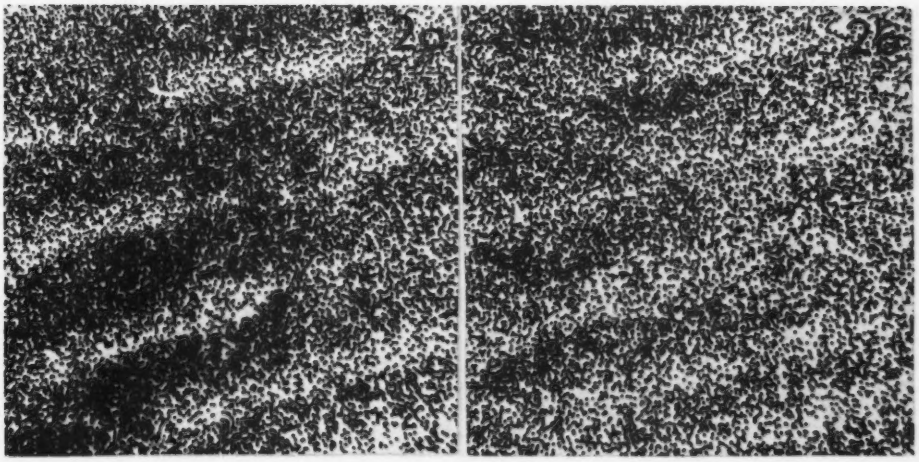

FIG. 2. Microphotographies réalisées lors d'un balayage microspectrométrique effectué dans la partie de l'écaille représentée à la Fig. 1 et ajusté pour le calcium (Fig. 2a à gauche) et pour le cuivre (Fig. 2b à droite). Les "circuli" de la Fig. 1 correspondent aux lignes claires sur les présentes microphotographies. Leur caractère clair sur celles-ci est dû au fait qu'ils ont émis peu de rayons X spécifiques du calcium ou du cuivre lorsqu'ils étaient soumis au faisceau électronique; ceci traduit leur pauvreté en calcium ou en cuivre. Par contre, les dépressions entre les "circuli" se révèlent riches en calcium ou en cuivre. On constate aussi que les points imprimés dus aux rayons X sont plus nombreux par unité de surface après un balayage microspectrométrique pour le calcium que pour le cuivre (comparer les Fig. 2a et 2b) alors que les conditions de balayage et de microphotographie avaient été identiques. G = 300 ×.

TABLEAU 1. Composition minérale par rapport au calcium pour les écailles du *Catostomus commersoni* vivant dans la rivière Manicougan et dans la rivière des Prairies.

Eléments	Rapports A Métaux/calcium rivière Manicouagan	Rapports B Métaux/calcium rivière des Prairies	Rapports B Rapports A
Ca/Ca	1.0000	1.0000	1.00
Cu/Ca	0.0250	0.0122	0.49
Na/Ca	0.0127	0.0099	0.78
K/Ca	0.0091	0.0076	0.83
Fe/Ca	0.0065	0.0056	0.86
Mg/Ca	0.0125	0.0114	0.91
Zn/Ca	0.0145	0.0132	0.91
Al/Ca	0.0049	0.0050	1.02
Ba/Ca	0.0046	0.0047	1.02
Sr/Ca	0.0073	0.0088	1.20
Mn/Ca	0.0024	0.0031	1.30
Cs/Ca	0.0011	0.0015	1.36
Cd/Ca	0.0016	0.0026	–.63
Ag/Ca	0.0021	0.0036	1.71
Hg/Ca	0.0019	0.0045	2.37
Cr/Ca	0.0019	0.0051	2.68
Co/Ca	0.0016	0.0049	2.81
Pb/Ca	0.0018	0.0052	2.89
Ni/Ca	0.0018	0.0054	3.00

TABLEAU 2. Caractéristiques minérales connues[a] pour A) la rivière Manicouagan, B) la rivière des Prairies.

Eléments	Eau		Sédiments[b]	
	A	B	A	B
Na	0.66 à 0.70 ppm	2.60 à 3.00 ppm	inconnu	inconnu
Mg	0.40 à 0.60 "	2.60 à 2.80 "	"	"
K	0.40 à 0.60 "	0.80 à 1.44 "	"	"
Ca	1.10 à 1.80 "	10.10 à 11.60 "	"	"
Cu	non décelable	6.00 à 20.00 ppb	7.00 à 26.00 ppm	45.00 à 194.00 ppm
Zn	" "	12.00 à 32.00 "	14.00 à 68.00 "	230.00 à 492.00 "
Mn	0.01 à 0.05 ppm	0.11 à 0.15 ppm	4.00 à 31.00 ppm	326.00 à 570.00 ppm
Co	non décelable	0.20 à 1.70 ppb	6.00 à 12.00 "	14.00 à 20.00 "
Ni	" "	1.00 à 2.50 "	8.00 à 18.00 "	32.00 à 78.00 "
Cd	" "	0.30 à 1.00 "	0.80 à 2.00 "	1.30 à 15.00 "
Hg	" "	2.00 à 15.00 "	0.02 à 0.11 "	0.57 à 3.00 "
Pb	" "	3.00 à 13.00 "	6.00 à 10.00 "	114.00 à 560.00 "

[a]Données fournies par l'institut national de la recherche scientifique, section eau, Université du Québec. Les quantités pour chaque métal varient avec le lieu et le moment de la prise dans les rivières tant pour l'eau que pour les sédiments.

[b]Le catostome étant un fouisseur de vase, on doit tenir compte de la composition minérale des premiers centimètres des sédiments à faible profondeur.

En ce qui concerne l'application de la microscopie électronique analytique aux écailles de poisson, nous démontrons dans une autre étude (van Coillie et al. 1973b) que, malgré la difficulté de préparation de standards uniformes de comparaison pour cette méthode, celle-ci s'avère plus précise et plus sensible que diverses autres méthodes telles que la spectrophotométrie à absorption atomique, l'histophotométrie ou la radioautographie. Dans le présent travail, l'analyse microspectrométrique apporte des précisions sur la composition et l'organisation minérales des écailles de poisson. En général, ces structures ostéoïdes contiennent, en poids sec, 40–60% de protéines de type fibreux et 40–60% de sels minéraux (van Oosten 1957: p. 230). Parmi ces derniers, en plus du calcium largement prédominant, on trouve du Na, Mg, Al, K, Fe, Cu, Zn et Ba (van Oosten 1957: p. 230, Snyder 1958; Cowgill et al. 1968; Love 1970: p. 259). Il en est de même pour les écailles du catostome mais celles-ci renferment en plus un peu de Cr, Mn, Co, Ni, Sr, Ag, Cd, Cs, Hg et Pb. Ceci est nouveau car on ne détecte pas normalement ces derniers métaux dans les écailles de poisson. S'agit-il de contaminations? C'est peu vraisemblable car nous avons décelé la même série d'éléments dans des sections ultrafines des écailles du catostome à l'aide de la microscopie électronique analytique par transmission. Tous les métaux précités doivent dès lors faire partie des sels minéraux non hydrosolubles ou être liés aux macromolécules au sein de ces structures ostéoïdes. En effet, la possibilité qu'on soit en présence d'éléments adsorbés à la surface de l'écaille, dispersés sous forme libre dans celle-ci ou intégrés à des annexes histologiques contaminant celle-ci est à rejeter puisque les écailles du catostome furent débarrassées de leurs tissus voisins et perdirent leurs éléments non spécifiquement intégrés lors de leur préparation histologique. Grâce au balayage microspectrométrique, il a également été possible d'établir que les métaux se situent surtout entre les circuli dans les écailles (Fig. 2a et 2b). Si l'on associe cette donnée à la texture fibreuse des circuli précisée ailleurs (van Coillie et al. 1973a) et aux caractéristiques histologiques et chimiques connues pour les écailles (van Oosten 1957: p. 225), on peut formuler l'hypothèse suivante concernant l'organisation histochimique de ces structures ostéoïdes: les circuli, pauvres en métaux, seraient principalement formés de protéines de type fibreux tandis que les dépressions entre ces circuli seraient surtout des plages d'accroissement minéral.

Composition minérale des écailles d'un poisson vivant dans des milieux différents: le renouvellement minéral au sein des écailles est normalement très lent car le calcium, "marqué" par des tétracyclines fluorescentes, reste durant plusieurs mois à son lieu de dépôt dans l'écaille (van Coillie 1967). Toutefois, le remplacement d'un métal par un autre peut s'effectuer rapidement dans les écailles comme l'indiquent les deux faits suivants (van Coillie et al. 1973a): (i) il ne faut pas plus de 3 h pour que du cadmium présent dans l'eau soit incorporé dans des écailles d'un cyprin tandis que le calcium y devient légèrement moins abondant; (ii) si des truites sont élevées dans des aquariums contenant 14 ou 24 ppb de cuivre au lieu de 4 ppb, leurs écailles acquièrent une teneur en cuivre amplifiée de 11 ou 18% respectivement au détriment du calcium. Ces deux observations soutiennent que, nonobstant la stabilité biochimique des écailles au point de vue minéral, la composition minérale de ces dernières change lorsque les conditions minérales du milieu de vie varient. C'est ce que nous avons voulu vérifier avec le *Catostomus commersoni* issu de deux milieux ayant des caractéristiques minérales différentes tout en n'ignorant pas la possibilité que la nourriture influence probablement aussi partiellement la composition minérale des structures ostéoïdes. Même si l'on ne dispose pas actuellement, pour l'eau et les sédiments des deux milieux considérés, de données quantitatives pour tous les métaux analysés dans les écailles du catostome, les valeurs signalées au Tableau 2 suffisent pour esquisser une comparaison entre la composition minérale du milieu de vie du catostome et celle des écailles de ce poisson (Tableau 1). Cette comparaison fournit les indications suivantes: (i) bien que le Na, Mg, K, Ca, Cu et Zn soient plus abondants dans la rivière des Prairies que dans la rivière Manicouagan, ces métaux sont moins abondants dans les écailles du catostome de la rivière des Prairies que dans celles du catostome de la Manicouagan; (ii) la prédominance de Mn, Co, Ni, Cd, Hg et Pb dans les écailles pour le catostome vivant dans la rivière des Prairies par rapport à celui issu de la Manicouagan est parallèle à la prépondérance de ces métaux dans le premier milieu vis-à-vis du second milieu. Devant ces constatations, il y a lieu de s'interroger sur la possibilité suivante: dans un milieu pollué par des quantités élevées de Mn, Co, Ni, Cd, Hg et Pb, le catostome accumule-t-il ces métaux dans ses écailles au détriment des éléments Na, Mg, K, Ca, Cu et Zn normalement présents au sein de ces structures ostéoïdes? Si tel est le cas, en emmagasinant des métaux toxiques, les écailles constitueraient alors un moyen physiologique indirect de résistance pour le poisson vis-à-vis des effets néfastes de ces derniers. On reste toutefois surpris que le Cu et le Zn, connus pour leur toxicité lorsque leur concentration devient importante,

soient plus abondants dans les écailles du catostome provenant de la Manicouagan que dans celles du même poisson pris dans la rivière Les Prairies En tant qu'éléments biologiques dans les écailles, ces deux métaux y sont-ils remplaçables par divers métaux toxiques et/ou, en tant qu'éléments toxique's y sont-ils moins incorporables que plusieurs autres métaux?

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Daily Feeding Rates of Bluegill (*Lepomis macrochirus*) Determined by a Refined Radioisotope Method

SEPPO E. KOLEHMAINEN[1]

Ecological Sciences Division
Oak Ridge National Laboratory, Oak Ridge, Tenn. USA

KOLEHMAINEN, S. E. 1974. Daily feeding rates of bluegill (*Lepomis macrochirus*) determined by a refined radioisotope method. J. Fish. Res. Board Can. 31: 67–74.

The daily values of ^{137}Cs intake by bluegill (*Lepomis macrochirus*) from a small impoundment receiving low-level radioactive effluents were used to calculate the daily feeding rates of the fish. The calculations of ^{137}Cs intake, for a nonequilibrium state, were based on fish growth, the seasonal fluctuation of ^{137}Cs concentration in fish, and the temperature-elimination rate relationship. The feeding rates were obtained by dividing the daily intake of ^{137}Cs by the concentration of ^{137}Cs in the diet. The daily meal of bluegill varied from 0.8% of body weight in February to 3.2% in June with an annual mean of 1.75%. There was a positive correlation ($r^2 = 0.805$) between the daily meal and the daily temperature. The ecological growth efficiency was 4.2% during the period from April to October.

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Nous avons utilisé les valeurs quotidiennes d'absorption du ^{137}Cs par le crapet arlequin (*Lepomis macrochirus*) dans un petit réservoir de retenue recevant des effluents à bas niveau de radioactivité pour calculer le taux quotidien d'alimentation des poissons. L'absorption du ^{137}Cs, dans un état de non-équilibre, a été calculée à partir de la croissance des poissons, des fluctuations saisonnières des concentrations de ^{137}Cs dans les poissons et de la relation température-élimination. On a déterminé les taux d'alimentation en divisant l'absorption quotidienne de ^{137}Cs par la concentration de ^{137}Cs dans le régime.

La ration quotidienne du crapet arlequin varie de 0.8% du poids corporel en février à 3.2% en juin, avec moyenne de 1.75%. Il y a corrélation positive ($r^2 = 0.805$) entre la ration et la température quotidiennes. L'efficacité écologique de croissance est de 4.2% durant la période avril–octobre.

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THIS paper describes a method of calculating the daily feeding rates of fish for any given time of the year by a refined radioisotope method. The fish used were bluegill (*Lepomis macrochirus*) in White Oak Lake, in a U.S. AEC Reservation, Oak Ridge, Tennessee. This small impoundment receives low-level radioactive wastes from Oak Ridge National Laboratory at a nearly constant rate. This made it possible to determine the uptake of ^{137}Cs in bluegill from season to season and then utilize the values of ^{137}Cs uptake for calculating the daily feeding rates for different times of the year.

Radiotracer techniques for measuring the feeding rates of animals in an equilibrium state under field conditions was originally suggested by Davis and Foster (1958). An equilibrium situation is not however, common in fish because, even though the concentration of the radioisotope in the environment may stay constant, growth processes of the fish cause the body burden of the radioisotope to increase with time.

The effect of growth on the body burden was taken into consideration by Kevern (1966) when he calculated the mean annual feeding rate in carp. Kevern treated the growth of carp as a constant process throughout the year, which makes the calculations simple but may cause a slight error in the feeding rates because growth occurs mostly in early summer, and the weight may even decrease in winter (Brown 1957; Gerking 1966). Kevern's method gives only the annual mean, but not the seasonal daily feeding rates.

In this study the feeding rates were calculated for a situation in which the following factors affecting the balance of the radioisotope were changing seasonally and were taken into consideration:

[1]Permanent address: Radioecology Division, Puerto Rico Nuclear Center, Mayaguez, Puerto Rico 00780.

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concentration of the radioisosope in the fish; growth of the fish; relationship between temperature and the elimination rates of the radioisotope; and concentration of the radioisotope in the food items of the fish, absorption of the radioisotope from different food items, and the proportion of different food items in the diet of the fish.

The data were collected from June 1967 to January 1969 in a field study of the mass balances of ^{137}Cs, stable cesium, and potassium in bluegill in White Oak Lake (Kolehmainen and Nelson 1969; Kolehmainen 1972).

^{137}Cs Budget of Bluegill

All calculations were based on average-sized bluegill belonging to age-group III during the first part of the year and to age-group IV during the last part of the year (Kolehmainen and Nelson 1969). Age of the bluegill was based on the growth year beginning at the time of annulus formation in the scale (early April in White Oak Lake).

Body Burden of ^{137}Cs

The body burden was determined by multiplying the concentration of ^{137}Cs in bluegill at time t by the weight of bluegill at time t (Fig. 1). The fish averaged 91.6 g (♀ 93.6 ± 19.4 (one standard deviation) and ♂ 89.6 ± 21.1) in January and gained 18.6 g by the next December (Kolehmainen and Nelson 1969). Ninety percent of the annual growth occurred during the period from April to November. Growth was assumed to follow a sigmoid curve (Gerking 1966) with a maximum growth rate in May and June. The weight of the fish in grams (m) on each day of the year was simulated (Fig. 1) with an empirical fit to a hypothetical sigmoid curve applying the arctan function

$$m = 7.19 \arctan [0.02 (t - 150)] + 100.7 \text{ g} \quad (1)$$

where t was the number of the day of the year, 150 was the day when half of the annual growth was assumed to be achieved (the inflection point), and 100.7 was the weight of the fish (in grams) on the 150th day. The seasonal cycle of ^{137}Cs concentration, c, in bluegill (33 samples totaling 206 fish, Fig. 2) was simulated (Fig. 1,2) with an empirical fit to a sine curve (Kolehmainen 1972),

$$c = 9.2 \sin \left[\frac{2\pi}{360} (t - 313)\right] + 38.2 \text{ pCi}^2\text{/g fresh wt}, \quad (2)$$

where t was the number of the day of the year, 313 was used to time the sinusoidal curve with the annual fluctuation, 9.2 was the fluctuation of the concentration above and below the geometrical annual mean concentration, 38.2 pCi/g fresh wt.

Elimination Rates of ^{137}Cs

Elimination of a radioisotope in organisms is usually expressed as a one- or multi-component exponential function (Fig. 3) at a given temperature, the different components being called biological half-lives or elimination rate constants. It is not known whether these different components reflect any specific metabolic functions, but they could represent certain phases in the absorption, distribution, deposition, and turn-over of the radioisotope. The percentage that is excreted in feces

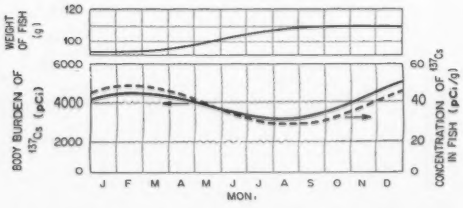

FIG. 1. Daily average weights of 74 bluegill during 1967–68 (uppermost curve) calculated by equation 1 (see text); concentrations of ^{137}Cs (pCi/g wet weight of fish; broken line), represented by a sine function, equation 2 and Fig. 2; and concentrations of ^{137}Cs in fish (lower solid line) calculated by multiplying the average concentration of ^{137}Cs in fish at time t (equation 2) by the weight of fish at time t (equation 1). All values (data of Kolehmainen and Nelson 1969) are calculated for average size White Oak Lake bluegill that were 4 years old in the summer.

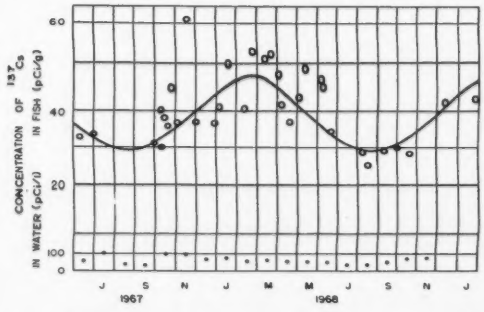

FIG. 2. Monthly concentrations of ^{137}Cs in White Oak Lake bluegill (pCi/g wet weight), and of ^{137}Cs in the water, June 1967–January 1969. The solid line is a sine wave calculated with a least squares method based on 33 samples totaling 206 fish (see equation 2). Figure reproduced from Health Physics 23 (1972), 301–315, by permission of the Health Physics Society.

[2]pCi (picocurie) is 10^{-12} curie or 2.22 disintegrations per minute.

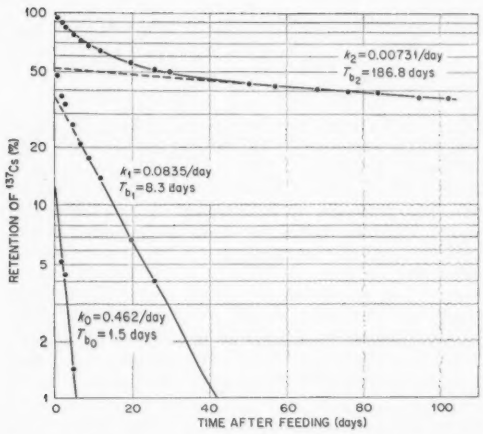

FIG. 3. Retention of ^{137}Cs in a 72 g bluegill after a single feeding (uppermost curve) and the elimination rate constants calculated with the "stripping method" (see text). Rate constant k_o represents elimination of unabsorbed ^{137}Cs from the alimentary canal. Rate constant k_1 is the fast component and k_2 the slow component of the elimination of absorbed ^{137}Cs. T_{b_0}, T_{b_1}, and T_{b_2} represent the different components of the total elimination, and are defined in terms of biological half-lives. For the mean values of the elimination rates of twelve bluegill see the text. Figure reproduced from Health Physics 23 (1972) 301–315, by permission of the Health Physics Society.

is the only part of the elimination that is a well-defined component. Otherwise the animals are usually treated as a "black box" when elimination is concerned.

Elimination rates of ^{137}Cs in bluegill were determined with 20 fish transferred from White Oak Lake to the laboratory, and with 12 fish that were given a single force-feeding of ^{137}Cs in a gelatin capsule. The retention of ^{137}Cs in these fish was counted at various time intervals (Kolehmainen 1972). The elimination rates were calculated by plotting the retention of ^{137}Cs in each fish on semilogarithmic paper. Figure 3 shows how the different components were determined by a "stripping method" (Comar 1955). First the slope of the straight part of the retention curve was calculated with a least-squares method and plotted in the curve. This slope gives the so-called slow component. The intercept of the line k_2 on the Y-axis gives the percentage of each single intake that is eliminated at the rate of the slow component. The corresponding values of this line were subtracted from the data points above it and these new values were plotted on the same graph paper. The points falling on a straight line gave the fast component (k_1). Accordingly, by subtracting the values of k_1 from the four points above the line of k_1, the points giving the slope of k_0 were obtained. The counting of ^{137}Cs in the fecal pellets confirmed that k_0 represents the elimination of unabsorbed ^{137}Cs in the feces. Since unabsorbed ^{137}Cs in food was not included in the body burden, k_0 is omitted in the calculations. In addition to the single-feeding experiment the slow component of the elimination process was determined in 20 other fish taken from White Oak Lake. No significant difference ($F = 0.12$) was observed in the slow component between the fish that received ^{137}Cs in a gelatin capsule and fish that were taken from White Oak Lake and that, presumably, were in a steady state with ^{137}Cs in their food (Kolehmainen 1972).

The fast rate of elimination, k_1, was 0.0912 ± 0.0054/day ($n = 12$) and the slow rate, k_2, was 0.00370 ± 0.00023/day ($n = 32$) at 15.8 C, the mean annual temperature of White Oak Lake. The elimination of ^{137}Cs in fish seems to follow the Q_{10} law (Kevern 1966; Häsänen et al. 1967, 1968). The percentage of the absorbed intake eliminated at k_1 (p_1) was $36.9 \pm 4.0\%$ and at k_2 (p_2) $63.1 \pm 4.0\%$. Values of p_1 and p_2 are probabilities that the absorbed ^{137}Cs atoms in each intake go into the corresponding compartment of the body burden, a_1Q and a_2Q. Radiocesium in the compartment a_1Q is eliminated at k_1 and in the compartment a_2Q at k_2. Values of a_1 and a_2 change according to the duration of feeding on food contaminated with the radioisotope. After a single intake of ^{137}Cs, $a_1 = p_1$ and $a_2 = p_2$, but under a continuous intake the value of a_1 decreases and, when the steady state is achieved, a_1Q forms only a small percentage of the body burden (Fig. 4). The values of a_1 and a_2 in an equilibrium state were calculated on the basis of p_1 and p_2 applying the equilibrium equation

$$Q_e = \frac{I}{k} \qquad (4)$$

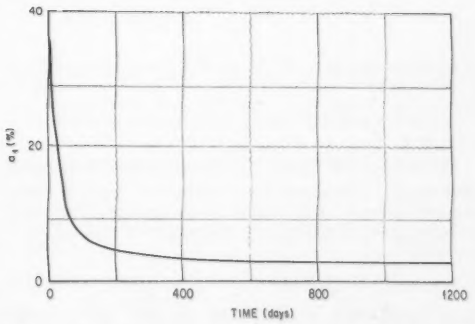

FIG. 4. Computerized daily percentages of the body burden, a_1, excreted as the fast component of the elimination process, k_1, in bluegill during continuous feeding on ^{137}Cs contaminated food. The elimination rate constants are $k_1 = 0.0912$/day and $k_2 = 0.00370$/day (see p. 69). The percentage of the intake (p_1) going to compartment a_1Q is 36.9% and that to compartment a_2Q is 63.1% (see p. 69). At equilibrium a_1 is 2.3% (see p. 70).

The equilibrium body burden (Q_e) contains two compartments such that $Q_e = a_1Q_e + a_2Q_e$. Correspondingly, the intake can be subdivided into two intakes: $I = p_1I + p_2I$. Now equation 4 can be rewritten

$$a_1Q_e = \frac{p_1I}{k_1}, \tag{5a}$$

and

$$a_2Q_e = \frac{p_2I}{k_2} \tag{5b}$$

When values of k_1, k_2, p_1 and p_2 were substituted in equation 5b, it gave

$$a_1Q_e = (4.05)\cdot(I) \tag{6a}$$

and

$$a_2Q_e = (170.1)\cdot(I) \tag{6b}$$

the total body burden being the sum of equations at 6a and 6b:

$$Q_e = (174.1)\cdot(I) \tag{7}$$

The body burden was 174.1 times the daily intake and $a_1 = 2.3\%$ and $a_2 = 97.7\%$ of the body burden (Fig. 4).

Daily Intake of ^{137}Cs

The basic formula for calculation of the intake of ^{137}Cs prior to an equilibrium is (Miettinen et al. 1963; *Kolehmainen et al. 1967; Reichle 1969):*

$$Q_t = Q_o\cdot e^{-kt} + \frac{I}{k}(1 - e^{-kt}) \tag{8}$$

where Q_t is the body burden of ^{137}Cs at time t and Q_o is the burden at time o (some previous time when analyzed). The intake I and the elimination rate coefficient k are considered constant during the period from time o to time t.

The intake of ^{137}Cs was calculated by modifying equation 8 for a two-component elimination process (since the stomach and gut contents were removed from fish before the counting of the body burden of ^{137}Cs, k_o was omitted):

$$Q_t = a_1Q_o\cdot e^{-k_1t} + a_2Q_o\cdot e^{-k_2t} + \frac{p_1I}{k_1}(1 - e^{-k_1t}) + \frac{p_2I}{k_2}(1 - e^{-k_2t}) \tag{9}$$

Since I, k_1, and k_2 were treated as constants during time o to time t, a short time, 1 day, was chosen as the time unit between the iterations. The daily intake was calculated then:

$$I = \frac{Q_t - (a_1Q_{t-1}\cdot e^{-k_1} + a_2Q_{t-1}\cdot e^{-k_2})}{\frac{p_1}{k_1}(1 - e^{-k_1}) + \frac{p_2}{k_2}(1 - e^{-k_2})} \tag{10}$$

Values of Q_t were calculated by multiplying the weight of bluegill (equation 1) by the concentration of ^{137}Cs in bluegill (equation 2, Fig. 1). The value of a_1 varied from 0.7 to 4.5% at different times of the year, but for *practical purposes, constant values of a_1 (2.3%) and* a_2 (97.7%) were used. Error in the intake caused by this simplification was less than ± 2%. No correction was made for the loss of ^{137}Cs in the reproductive cells (about 5% in females and 1% in males). Elimination rates were assumed to double with an increase of 10 C in the temperature (Kevern 1966; Häsänen et al. 1967, 1968; Ulrikson et al. 1970). The equation for the temperature relationship of the elimination rates (Q_{10} = 2) was:

$$k_t = k_{15.8}\, e^{0.0693\left\{[\sin\frac{2\pi}{360}\cdot(t-105)]\cdot[10.8]\right\}} \tag{11}$$

where k_t was the value of k at time t, $k_{15.8}$ was the value of k at the mean annual temperature of White Oak Lake (15.8 C), t was the number of the day of the year, 105 was the number of the day when water temperature in the spring reached 15.8 C, and 10.8 was the fluctuation of the temperature above and below the mean annual temperature. The values of k_2 are given in Fig. 5.

Values of the weight of fish (equation 1), the concentration of ^{137}Cs in fish (equation2), the body burden Q_t and Q_{t-1} (equation 1 times equation 2), k_1 and k_2 (equation 11), and the daily intake of ^{137}Cs (equation 10) were calculated step-wise with a computer program. The intake of ^{137}Cs in fish fluctuated from 0.065 pCi/g wet weight in late March to 0.334 pCi/g in the middle of August (Fig. 5). The annual mean intake was 0.256 pCi/g of fish. Fluctuations in the intake rates were caused by

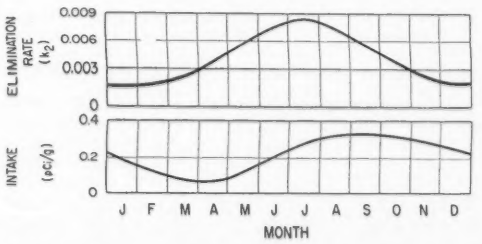

FIG. 5. Calculated daily values of the elimination rate constant k_2 and daily intake of ^{137}Cs during the year 1968 assuming that k_2 followed the relationship Q_{10} = 2 (equation 11 using the mean k_2 of 32 fish at 15.8 C). The daily intake (pCi/g wet weight of fish) was calculated on the basis of the body burden of 206 fish (Fig. 1) and the elimination rate constants k_1 and k_2 (equation 10) for White Oak Lake bluegills that were 4 years old in the summer.

fluctuations in the concentration of ^{137}Cs in the food items and the feeding rates of bluegill (Kolehmainen and Nelson 1969; Kolehmainen 1972).

Daily Feeding Rates of Bluegill

Feeding rates of bluegill were calculated by dividing the daily intake of ^{137}Cs by the concentration of ^{137}Cs in the diet. The formula for the daily food intake, r', was (Kevern 1966):

$$r' = \frac{I}{b_i \cdot d_i \cdot f_i} \quad (12)$$

where I was the daily intake of ^{137}Cs, b_i the absorption percentage of ^{137}Cs for the ith food item, d_i the concentration of ^{137}Cs in the ith food item, and f_i the proportion of ith food item in the diet of bluegill.

Composition of the bluegill diet during a year was determined on the basis of 3–15 stomach samples collected monthly (total 153) between September 1967 and September 1968. Proportions of different food items in the stomach contents were estimated visually under a microscope. Weighing some stomach samples after different food items had been separated into groups showed good agreement between the visual and gravimetric estimations.

The average percentages of the Main Food Items by volume in the 153 stomachs during the year were:

Food item	Percentage
Chironomid larvae	56.1
Chironomid pupae and adults	6.4
Other insects: larvae, pupae, and adults	*14.4*
Fish eggs	3.5
Fish	0.9
Plant material	4.9
Detritus and sediments	9.6
Others	4.2
	100.0

Absorption of ^{137}Cs from different food items of White Oak Lake bluegill was determined experimentally. When fish were fed chironomid larvae, 16% of the ^{137}Cs in the larvae was absorbed; from algae, fish, and fish eggs, 69% was absorbed, and from detritus, 3% (Kolehmainen 1972). Absorption of ^{137}Cs for other insect larvae was assumed to be the same as for chironomid larvae. Concentrations of ^{137}Cs in different food items (Table 1) were determined by two methods: by counting the radioactivity in stomach contents of the fish, and by calculating the sum of the proportion of each food item, f_i, times the concentration of ^{137}Cs in the same food items on the same date, d_i (Table 2). Some values of d_i had to be interpolated. Observed (γ-counted) and calculated ($d_i f_i$) concentrations of ^{137}Cs in the stomach contents agreed well. The calculated values were used.

The feeding rates were calculated with the equation (12) as in the following example:

On June 4, 1968, the calculated intake, I, of ^{137}Cs was 0.168 pCi/g of fish. Stomach contents of bluegill consisted of 50% chironomid larvae, 20% other insect larvae, 11% plants, and 19% detritus. The absorbed quantity of ^{137}Cs for 1 g of food was:

Food Item	b_i %	d_i pCi/g of food	f_i %	$b_i d_i f_i$ pCi
Chironomid larvae	16	40	50	3.2
Other insect larvae	16	28	20	0.9
Plants	69	14	11	1.0
Detritus	3	221	19	1.3
			per gram	6.4

A bluegill's daily food consumption was:

$$r' = \frac{0.168 \text{ pCi/g of fish}}{6.4 \text{ pCi/g of food}} = 0.0263 \text{ g food/g of fish} \quad (13)$$

which gives a daily meal of 2.63% of the body weight.

TABLE 1. Concentrations of ^{137}Cs (pCi per gram wet weight) in different food items of White Oak Lake bluegill collected by dredging.

Date	Aquatic insect larvae Chironomids	Others	Algae	Terrestrial insects	Fish eggs	Detritus	Other bottom animals
Dec. 15, 1967	105.7					381.7	
Dec. 22, 1967	123.2	20.2	49.8				
Mar. 28, 1968	55.2	18.4	23.4			190.4	
June 3, 1968			13.7		20.5	221.3	
July 16, 1968	72.4						39.7
Aug. 14, 1968				17.9			
Sept. 9, 1968		58.1	39.8				
Sept. 27, 1968	125.3		48.1			245.8	
Avg and SD	96.4±28.0	32.3±18.3	35.0±14.2	17.9	20.5	259.8±73.0	39.7

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Daily intakes of ^{137}Cs and absorbable concentrations of ^{137}Cs in bluegill's food on 15 occasions are given in Table 2.

There was a positive correlation between the daily meal of bluegill and the daily temperature of water in White Oak Lake. The linear regression between the feeding rates and the daily temperature was $Y = 0.40 + 0.076\ X$ ($r^2 = 0.805$) where Y was the daily meal in percent of the body weight and X the temperature in C. The daily meal was at a minimum (0.84% of the body weight) in February and increased slowly during March and April (Fig. 6). During May the daily meal increased rapidly toward a maximum (3.24%) in June, after which there was a gradual decrease until February. The daily meal for the whole year averaged 1.75% of the body weight. Food consumption of bluegill was calculated also with the balances of stable cesium and potassium. These results agreed well with the calculations based on ^{137}Cs balance (Kolehmainen and Nelson 1969).

There were four possible sources of errors in the calculations of the feeding rates: (1) the excretion rates of cesium were assumed to follow the so-called Q_{10} law, i.e., to double with an increase of 10 C in the temperature. This has not been proved for the biokinetic range of any species of fish, but it has been shown to be true for bluegill between 15 and 25 C (Ulrikson et al. 1970). (2) The numbers of ^{137}Cs analyses of different food items and stomach contents were small. (3) Assimilation of ^{137}Cs for minor food items was not determined. (4) The loss of ^{137}Cs in genital products was omitted.

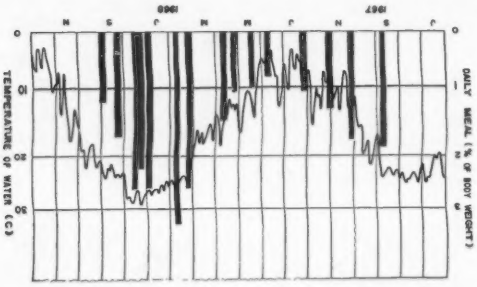

FIG. 6. Daily meals on 15 occasions from September 1967 to September 1968 of bluegill that were 4 years old in summer (bars) and the temperature of water in White Oak Lake (curve).

Discussion and Conclusions

The advantage of the radiotracer method in determining the feeding rates of fish is that it allows one to study the feeding rates in the field, thus minimizing the errors caused by the handling of fish in the laboratory. The only time in the radiotracer method when live fish have to be handled, either in the field or in a laboratory, is when the elimination rates are studied. One serious limitation of the radioisotope method is that there are only a few places where suitable radioisotopes can be found in natural waters. It is not, however, necessary to have radioisotopes in the water body in order to use the technique for feeding rate studies. Certain stable elements

TABLE 2. ^{137}Cs concentrations in stomach contents, gamma-counted and calculated ($d_i f_i$), daily assimilated intake of ^{137}Cs (I), and the quantities of ^{137}Cs absorbed from wet weight of stomach contents ($a_i d_i f_i$).

Date	^{137}Cs in stomach contents: Gamma-counted pCi/g	^{137}Cs in stomach contents: $d_i f_i$ pCi/g	Intake of ^{137}Cs I pCi/g fish	^{137}Cs absorbed from food $a_i d_i f_i$ pCi/g	No. stomachs
Sept. 29, 1967	81.3	74.0	0.333	18.0	11
Nov. 2, 1967	100.4	102.5	0.332	19.0	3
Dec. 1, 1967	105.1	101.0	0.283	21.9	4
Jan. 4, 1968	148.6	160.5	0.216	20.7	4
Feb. 20, 1968	109.4	115.5	0.111	13.3	3
Mar. 11, 1968	74.1	70.1	0.076	7.5	2
Apr. 5, 1968	79.6	84.0	0.065	6.2	14
Apr. 18, 1968	65.7	65.7	0.071	5.0	5
June 4, 1968	68.0	69.0	0.168	6.4	7
June 20, 1968	60.9	58.5	0.220	6.8	4
July 26, 1968	55.6	59.1	0.305	11.5	2
Aug. 6, 1968	58.4	60.7	0.315	14.0	12
Aug. 14, 1968	17.9	17.9	0.323	12.2	4
Sept. 7, 1968	85.1	81.1	0.333	19.7	5
Sept. 27, 1968	87.3	79.5	0.333	27.4	5
	79.9	80.7	0.233	13.5	Avg/Σ 84
SD	28.7	30.0	0.106	6.7	

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that are taken up by fish in the diet can be used as well. In that case the body burden of the element is determined in the fish and the concentration of the element in the diet of the fish. Absorption of the element from the food items and the elimination rates of the element in the fish can be studied in laboratory experiments with a radioisotope of the element. Suitable elements taken up by fish in their food are K, Rb, Cs, and many others. Before any particular element can be used for this purpose, one has to determine what percentage of the element is taken up directly from water. In the case of cesium the uptake from fresh water by fish is less than 1% (Garder and Skulberg 1966; Kolehmainen et al. 1967). Note that this percentage does not have to be the same in freshwater and marine fish.

An attempt was made to obtain seasonal values because until now there has been very little data on the feeding rates of fish outside the summer months. In laboratory experiments it has been shown that the feeding rates of fishes increase with temperature until an optimum temperature is reached, after which they again decrease (Pentelow 1939; Ricker 1946; Baldwin 1957; Kinne 1960; Warren and Davis 1967). In White Oak Lake bluegill the daily feeding rates were positively correlated ($r^2 = 0.805$) with the daily temperature of water. However, the temperature is not the only factor affecting the feeding rates on a seasonal basis, as fish are undergoing continuous adaptation processes seasonally and many species are known to cease feeding during the spawning season. Therefore, it is apparent that feeding rates of bluegill are only partly correlated with temperature on a seasonal basis.

The mean annual daily meal calculated with this method (1.75% of body weight) was similar to the value calculated by Kevern's (1966) method (1.73%), but was lower (1.62%) when calculated with the basic equilibrium equation as applied by Davis and Foster (1958). The advantage of Kevern's (1966) method over the basic method (Davis and Foster 1958) is that it takes into consideration the food consumed for the annual growth. The method described in this paper allows one to simulate the growth of fish seasonally on a daily basis and to calculate the daily meal of fish for any given time of the year. However, to use this method the composition of the diet, the concentration of the radioisotope in the food items, and the growth of the fish have to be studied on a seasonal basis.

The maximum daily meal of bluegill in age-group IV in White Oak Lake (3.2% of body weight) was somewhat lower than that of bluegill (3.6%) observed under laboratory conditions (Gerking 1962). Daily meals of White Oak Lake bluegill in June (2.9%) were close to those determined by Keast and Welsh (1968) for bluegill in Lake Opinicon, Ontario, (2.5%). For June to September, the value (2.0%) was somewhat higher than Seaburg and Moyle (1964) found for the same period for bluegill in Minnesota (1–2%).

The ecological growth efficiency (growth (g) food consumption (g) during the same period) in the age group IV in White Oak Lake bluegill for the period from April to October was 4.2%, wich is somewhat lower than that determined by Gerking (1962) for the same age group in Wyland Lake in Minnesota for the period from May to October.

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In Vitro and In Vivo Efficacy of a Potentiated Sulfonamide in Control of Furunculosis in Salmonids

G. L. Bullock, H. M. Stuckey, Diane Collis, R. L. Herman

Eastern Fish Disease Laboratory
Leetown, Route #1, Kearneysville, W. Va. 25430, USA

and G. Maestrone

Hoffmann-La Roche, Inc., Nutley, N.J. 07110, USA

Bullock, G. L., H. M. Stuckey, D. Collis, R. L. Herman, and G. Maestrone. 1974. In vitro and in vivo efficacy of a potentiated sulfonamide in control of furunculosis in salmonids. J. Fish. Res. Board Can. 31: 75–82.

In vitro and in vivo efficacy of a potentiated sulffonamide, Ro5–0037, for inhibition of *Aeromonas salmonicida*, causative agent of fish furunculosis was determined. The components of the potentiated sulfonamide, sulfadimethoxine, and ormetoprim were tested alone and in combination. In vitro results showed that both sulfonamide-resistant and sensitive strains of *A. salmonicida* had greater sensitivity to Ro5–0037 than to either of its components. Also, sulfonamide-sensitive strains developed resistance to ormetoprim and sulfadimethoxine more rapidly than to Ro5–0037. Efficacy studies with small numbers of artificially infected trout revealed that Ro5–0037 effectively controlled furunculosis at 50 mg/kg of fish/day, one fourth the usual sulfonamide dosage level. Extensive field trials also showed the 50 mg/kg level of Ro5–0037 gave effective control of hatchery outbreaks of furunculosis. As found in the in vitro studies, all in vivo trials indicated the combination of ormetoprim and sulfadimethoxine was more efficient in inhibiting *A. salmonicida* than either component alone.

Bullock, G. L., H. M. Stuckey, D. Collis, R. L. Herman, and G. Maestrone. 1974. In vitro and in vivo efficacy of a potentiated sulfonamide in control of furunculosis in salmonids. J. Fish. Res. Board Can. 31: 75–82.

Nous avons mesuré l'efficacité in vitro et in vivo d'un sulfamide renforcé, Ro5–0037, contre *Aeromonas salmonicida*, agent responsable de la furonculose des poissons. Les composants du sulfamide renforcé, le sulfadiméthoxine et l'ormétoprime, ont été essayés seuls et en combinaison. Les résultats in vitro démontrent que les lignées d'*A. salmonicida* résistantes aussi bien que sensibles au sulfamide ont une sensibilité plus grande au Ro5–0037 qu'à l'un ou l'autre de ses composants. De plus, les lignées résistantes au sulfamide développent une résistance à l'ormétoprime et au sulfadiméthoxine plus rapidement qu'au Ro5–0037. Des études sur un petit nombre de truites infectées artificiellement démontrent que Ro5–0037 contrôle efficacement la furonculose à un dosage de 50 mg/kg de poisson/jour, soit un quart du dosage usuel de sulfamide. Des essais poussés effectuées sur le terrain indiquent que le niveau de 50 mg/kg de Ro5–0037 contrôle efficacement les épidémies de furonculose dans les établissements de pisciculture. Comme le démontrent les études in vitro, tous les essais in vivo prouvent que la combinaison ormétoprime-sulfadiméthoxine contrôle plus efficacement *A. salmonicida* que l'un ou l'autre composant utilisé seul.

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The use of sulfonamides for treatment of systemic bacterial infections in salmonids dates to 1937 with the use of sulfanilamide on brook trout (*Salvelinus fontinalis*) infected with *Hemophilus piscium*, cause of ulcer disease (Tunison and McCay 1937). Since that time, various sulfonamides have been tested for controlling bacterial fish diseases, but presently only sulfamerazine has been approved by the United States Food and Drug Administration and only for treating hatchery salmonids suffering from furunculosis (Herman 1970). Sulfonamide-resistant strains of *Aeromonas salmonicida*, the cause of fish furunculosis, are known to occur, and sulfamerazine will not control all outbreaks of the disease. The clearance of oxytetracycline in 1970 for use in treating furunculosis and other

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bacterial diseases has facilitated treatment of furunculosis caused by sulfonamide-resistant strains of *A. salmonicida*.

Since strains of *A. salmonicida* as well as other bacterial fish pathogens may become resistant to both sulfamerazine and oxytetracycline, it is desirable to have additional drugs for treating systemic bacterial diseases. The present work describes the results of in vitro and in vivo testing of Ro5-0037, a drug containing a 5:1 ratio of sulfadimethoxine and ormetoprim, a folic acid antagonist, against *A. salmonicida*. Ormetoprim has been termed a potentiator since the combination of sulfadimethoxine and ormetoprim results in enhanced broad spectrum antibacterial and anticoccidial activity in chickens and turkeys (Marusich et al. 1969; Mitrovic et al. 1971a; Mitrovic et al. 1971b).

Materials and Methods

Experimental Drugs

The potentiated sulfonamide Ro5–0037 containing sulfadimethoxine (2,4-dimethoxy-6-sulfanilamido-1, 3 diazine) and ormetoprim (2,4 Diamino-5-(4,4 dimethoxy-2-methylbenzyl) pyrimidine) at a 5:1 ratio was used for this work. Samples of Ro5–0037, sulfadimethoxine and ormetoprim were furnished by Hoffmann-La Roche Inc., Nutley, N. J., for all experimental work.

In Vitro Tests

Twenty-three strains of *Aeromonas salmonicida*, maintained at the Eastern Fish Disease Laboratory (EFDL), were tested for sensitivity to Ro5–0037 and its components (Table 1). Using sulfamerazine sensitivity discs, each containing 1.0 mg of drug, 12 *A. salmonicida* strains had been found sulfonamide-sensitive and 11 sulfonamide-resistant.

In the present work all sensitivity determinations were made using a tube dilution technique employing 16 × 150 mm tubes and Mueller Hinton (M-H) broth (Difco) tests were performed as follows: (1) A stock solution containing 40,000 μg/ml of Ro5–0037 or ormetoprim was prepared in a mixture of 1 part 8.5% lactic acid and 1.6 parts of 100% dimethylsulfoxide (DMSO). (2) A 40,000 μg/ml stock solution of sulfadimethoxine was prepared in the same manner except using 100% DMSO as the solvent. (3) Stock solutions were diluted to 5000 μg/ml in warmed (50 C) M-H broth, and then further diluted in M-H broth to obtain test concentrations of 500, 100, 20, 4.0, 0.8, 0.16, and 0.032 μg/ml. A final 5 ml volume was contained in each tube.

Tubes and diluent medium used in preparation of stock and test solutions were sterile. However, the 40,000 μg/ml solutions were prepared using unsterilized lactic acid and DMSO. Since uninoculated stocks and controls remained sterile for at least a week after preparation, these substances were considered free from living microorganisms.

Each test strain of *A. salmonicida* was grown in M-H broth 24 hr at 20–22 C, diluted 1:200 or 1:400 and 0.1 ml of diluted culture inoculated into each test concentration. The same amount of inoculum was also placed into 5 ml M-H broth to test viability (inoculum control) and into M-H broth containing the same concentration of lactic acid-DMSO as found in 500 μg/ml concentration of Ro5–0037 or ormetoprim (lactic acid-DMSO control). This latter control was used to exclude the possibility of any inhibitory effect of the solvents on growth of the *A. salmonicida* strains. Inoculum for each test concentration

TABLE 1. *Aeromonas salmonicida* strains used in in vitro sensitivity tests of Ro5–0037, sulfadimethoxine, and ormetoprim.

Strain	Source and date of isolation
3.9, 3.12	Brown trout (*Salmo trutta*), W. Va., 1955
3.11, 3.15	Brook trout (*Salvelinus fontinalis*), W. Va., 1955
3.16, 3.17	Unknown
3.21	Brook trout, Wisconsin, 1956
3.22	Brook trout, W. Va., 1956
3.23	Brook trout, Pa., 1960
3.24	Brown trout, W. Va., 1960
3.26	Brook trout, W. Va., 1960
3.27	Brook trout, Md., 1960
3.28	Atlantic salmon (*Salmo salar*), Canada, 1958
3.30	Brown trout, W. Va., 1961
3.31	Chestnut lamprey (*Ichthyomyzon castaneus*), Mich., 1960
3.32	Brook trout, Md., 1961
3.33	Brook trout, W. Va., 1969
3.36	Brown trout, W. Va., 1970
3.43, 3.44, 3.47	Brown trout, Md., 1970
3.45, 3.46	Brown trout, S. C., 1970

or controls contained $1.8–3.9 \times 10^5$ viable *A. salmonicida* cells as determined by spread plate technique.

Tubes were incubated at 20–22 C and results recorded after 24 and 48 hr. Growth was scored as: – for absence of turbidity, + for slight turbidity, and ++ for heavy turbidity. The sensitivity of each culture to the three test compounds was determined at least twice.

Induction of in Vitro Resistance

After the in vitro sensitivity of *A. salmonicida* strains had been established for each of the test drugs, further studies were carried out with three sulfonamide-sensitive strains to determine how rapidly *A. salmonicida* would develop resistance to Ro5–0037 and its components. After the minimum inhibitory concentration (MIC) in μg/ml was established for each strain with each of the test drugs, a set of 10–15 tubes (final volume 10 ml/tube) M-H broth was prepared containing 0.25 MIC (1/4 MIC) for each of the test strains. This meant that each of the three strains had three sets of M-H broth with each set containing 1/4 MIC Ro5–0037, sulfadimethoxine, or ormetoprim. Each strain was grown 24 hr at 20 C in M-H broth and 0.1 ml inoculated into the first tube of each of the three sets of 1/4 MIC drug-broth mixtures. When growth occurred (usually within 24 hr) in the first tube of each set, 0.1 ml was transferred to the second tube of each of the three sets. This serial transfer was continued with each strain and drug for 9–11 passages. The MIC of each strain to each drug was again determined to detect possible increase in resistance. If resistance had not increased, additional sets were prepared and another 9–11 passages in the 1/4 MIC drug-broth mixture was carried out, followed by determination of the MIC. Strains were passaged up to 30 times in an attempt to induce resistance.

In Vivo Tests

The efficacy of Ro5–0037 in controlling furunculosis was determined under laboratory conditions by using artificially infected rainbow trout kept in 40–60-liter aquaria and also in hatchery raceways containing fingerling brook or brown (*Salmo trutta*) trout experiencing natural outbreaks of furunculosis.

Aquarium studies were conducted at the EFDL and also the Western Fish Disease Laboratory (WDFL) by Dr G. Wedemeyer. The aquarium studies were designed to obtain the following information: optimum dose level of Ro5–0037; effect of feeding optimum dose level for 3, 6, 9, 12, or 14 days; efficacy of Ro5–0037 as compared to that of its components, ormetoprim and sulfadimethoxine, and also of sulfisoxasole.

In aquarium studies conducted at the EFDL, fingerling and yearling trout were used. Two, 40–60-liter aquaria each containing 25 fingerling (average weight 39 g) or 10 yearling trout (average weight 106 g), were used for each test condition or nonmedicated controls. Experimental trout were allowed to acclimate 5–10 days in aquaria before the experiment was begun. Spring water at a constant temperature of 12.5 C supplied aquaria.

Furunculosis was experimentally induced in test trout by injecting *A. salmonicida* grown for 24 hr at 20–22 C on a slant of tryptic soy agar (Difco), suspended in nutrient broth, and adjusted to 89% transmittance at 525 nm. Each trout was injected intraperitoneally with 2×10^6 viable cells contained in .05 ml of the suspension. The first mortalities usually occurred within 96 hr after injection.

Drugs were coated onto pelleted diets using a 5.0% gelatin suspension as a binder. Because injection of large numbers of *A. salmonicida* might mask efficacy of the test drugs, medication was begun 1 day prior to injection.

Drug levels tested were: ormetoprim at 40 and 50 mg/kg of trout/day; sulfadimethoxine or sulfisoxasole at 200 mg/kg of trout/day; Ro5–0037 at 3.0, 6.0, 12.5, 25, 50, and 75 mg/kg of trout/day. Levels of Ro5–0037 above 75 mg were usually rejected by trout. Except where various treatment periods were tested, all medicated diets and nonmedicated control diets were fed to the trout for a 2-week period. The trout were then observed for an additional 4 weeks.

Procedures for the in vivo efficacy studies conducted at the WFDL were the same as those used at the EFDL except that experiments were carried for only 14 days after the medication period was completed, small (60–90 mm) trout were used, and each aquarium contained 15–25 trout.

Raceway efficacy trials were conducted at several state and federal hatcheries to test the effectiveness of Ro5–0037 in hatchery trout experiencing natural outbreaks of furunculosis. Through the cooperation of the Food and Drug Administration and state and federal personnel, raceway studies using fingerling brown or brook trout (10–20 cm) were conducted at Lamar National Fish Hatchery (NFH), Lamar, Pennsylvania; Leetown NFH, Kearneysville, West Virginia; Spearfish Fisheries Center (NFH), Spearfish, South Dakota; and Bear Creek Rearing Station (state of Maryland), Accident, Maryland.

When the presence of *A. salmonicida* was confirmed by slide agglutination tests using specific antiserum, replicated lots of trout were set up by partitioning raceways or moving trout to smaller raceways. A medicated diet containing Ro5–0037 at 50 mg/kg of trout/day was then prepared by combining an oyster shell premix containing 30% Ro5–0037 onto the proper size and amount of pellets (rate of 7.6 g premix per 100 lb trout/day) with a 5% gelatin suspension or oil (A and D feeding oil, corn oil, etc.). In one trial the drug was incorporated into the pellets. Sufficient medicated diet was prepared to last 3–7 days and samples of drug-coated diets were analyzed to determine the drug levels present on or in the pellets.

Trout received the 50 mg/kg per day medicated diet for 14 days and then were observed for at least an additional 14 days. Nonmedicated control trout were kept in each raceway trial. In raceway efficacy trials, approximately 53,000 (2460 kg) fingerling brook or brown trout received Ro5–0037 while 29,300 (1250 kg) fingerlings served as nonmedicated controls. In all trials moribund trout were examined periodically for presence of *A. salmonicida*. Trials were conducted between June and December, 1970. Water temperature ranged from 12–19 C.

Results

In Vitro Studies

After 24 hr incubation both sulfonamide-sensitive and resistant strains of *A. salmonicida* showed greater sensitivity to Ro5-0037 than to either of its components (Table 2 and 3). Continued incubation resulted in a slight increase in resistance to Ro5–0037 among sensitive strains and complete resistance (500 μg/ml MIC) to Ro5–0037 among 5 of 11 sulfonamide-resistant cultures.

Serial passages of three sulfonamide-sensitive strains showed that resistance developed more rapidly to ormetoprim and sulfadimethoxine than to Ro5–0037. Development of drug resistance in one of these strains is shown in Fig. 1. Results for the other two strains were similar except that one strain failed to develop resistance to Ro5–0037 even after 30 passages and both strains required 18–30 passages to show resistance to ormetoprim. In all in vitro tests, growth occurred in inoculum and lactic acid-DMSO control, showing viability of inocula and no inhibitory effects of lactic acid-DMSO.

In Vivo Studies

Aquarium studies at the EFDL showed Ro5–0037 at 50 mg/kg per day (one-fourth the recommended

Table 2. In vitro sensitivity to Ro5–0037, sulfadimethoxine, and ormetoprim of sulfonamide-sensitive strains of *Aeromonas salmonicida* (24 and 48 hr results).

	Minimum inhibitory concentration in μg/ml					
	Ro5–0037		Sulfadimethoxine		Ormetoprim	
Strain	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
3.9	0.8	4.0	4.0–20.0	4.0–100.0	0.8–20.0	4.0–20.0
3.11	0.8	0.8–4.0	4.0	4.0	0.8–4.0	4.0
3.16	0.8	0.8–4.0	4.0	4.0	4.0	4.0
3.21	0.16	0.16	0.8	0.8	0.8	0.8
3.22	0.8	0.8	4.0	4.0	4.0	100.0
3.23	0.8–4.0	4.0–20.0	20.0–100.0	20.0–100.0	4.0–20.0	500.0
3.27	0.16	0.16–0.8	0.8	4.0	0.8	4.0
3.35	0.8	0.8	4.0	4.0	0.8	4.0
3.36	0.8	0.8	0.8–4.0	20.0	4.0	4.0
3.43	0.8	0.8	0.8	4.0	0.8	0.8
3.44	0.8	0.8	4.0	4.0	0.8	4.0
3.47	0.8	0.8	4.0	4.0	0.8	4.0

Table 3. In vitro sensitivity to Ro5–0037, sulfadimethoxine, and ormetoprim of sulfonamide-resistant strains of *Aeromonas salmonicida* (24 and 48 hr results).

	Minimum inhibitory concentration in μg/ml					
	Ro5–0037		Sulfadimethoxine		Ormetoprim	
Strain	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
3.12	4.0–20.0	500.0	500.0	500.0	20.0–100.0	500.0
3.15	4.0	500.0	500.0	500.0	20.0	500.0
3.17	4.0	100.0	500.0	500.0	20.0	100.0
3.24	0.8–4.0	4.0–20.0	500.0	500.0	4.0	20.0
3.26	0.8	4.0	500.0	500.0	4.0	20.0
3.28	20.0	500.0	500.0	500.0	100.0	500.0
3.30	0.8–4.0	500.0	500.0	500.0	20.0	500.0
3.31	4.0	500.0	500.0	500.0	4.0	500.0
3.32	0.8	4.0	100.0–500.0	500.0–>500.0	4.0	4.0
3.45	4.0	4.0	500.0	500.0	4.0	500.0
3.46	0.032	4.0	0.032	500.0	0.032	500.0

sulfonamide dose level), and sulfadimethoxine and sulfisoxazole at 200 mg/kg per day all controlled furunculosis in experimentally infected rainbow trout (Fig. 2). Levels of Ro5–0037 below 50 mg/kg did not adequately control experimental *A. salmonicida* infections (Fig. 3), but as little as 3 days of feeding the 50 mg/kg of trout/day level effectively controlled experimental infection. The 50 mg/kg level of Ro5–0037 was also more effective in furunculosis control than either of its components alone, even though both the sulfadimethoxine (200 mg/kg per day) and ormetoprim (50 mg/kg per day) levels fed were five and six times greater respectively than their levels in 50 mg/kg per day of Ro5–0037 (Fig. 4).

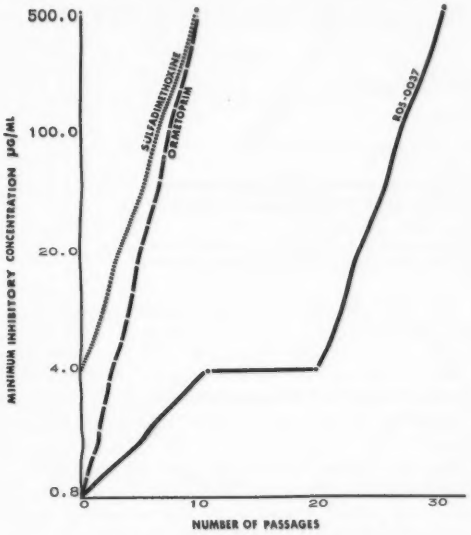

FIG. 1. Effect of repeated passaging of a sulfonamide sensitive strain of *Aeromonas salmonicida* in one-fourth the minimal inhibitory concentration of Ro5–0037, and its components.

Aquarium studies conducted at the WFDL on effective levels of Ro5–0037 and comparison of Ro5–0037 and its components for control of furunculosis confirmed results obtained at the EFDL.

The 50 mg/kg per day level of Ro5–0037 was also effective in controlling hatchery outbreaks of furunculosis. All *A. salmonicida* strains isolated from moribund fish from each of the raceway trials were found to be sensitive to sulfonamides. Typical results are presented in Fig. 5 and 6. As seen in these figures, mortality among nontreated controls continued to rise during the experimental period, while mortality in treated lots was reduced during the treatment period and did not rise during the 2–3-week observation period. The only exception was the efficacy trial involving brook trout at Leetown NFH. Two weeks following the end of treatment mortality gradually increased in treated lots and was still increasing when the trial was completed. These trout were anemic prior to treatment due to nephrocalcinosis (Landolt 1972), which presumably weakened them.

Analysis of medicated diets used in the efficacd trials showed that all but one of the diets containey between 88–110% of the calculated amount of Ro5–

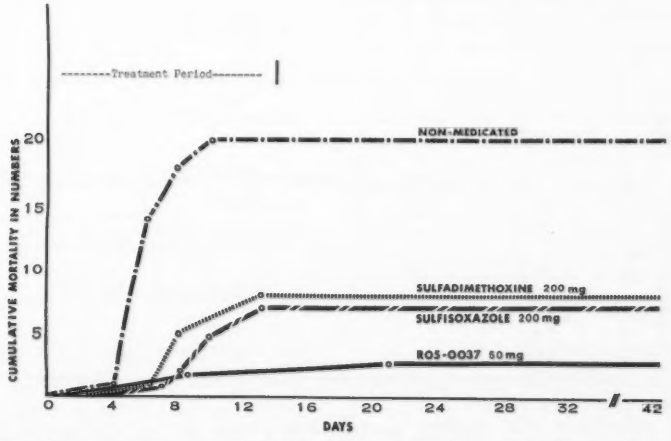

FIG. 2. Comparison of Ro5–0037, sulfadimethoxine, and sulfisoxazole in controlling experimentally-induced furunculosis in rainbow trout (*Salmo gairdneri*). Results from replicate aquaria have been combined.

0037. The Ro5–0037 gelatin-coated diet fed at Spearfish NFH contained only 75% of the calculated drug (37.5 mg/kg of trout/day) but was still effective (Fig. 6). Also, as shown in Fig. 6, Ro5–0037 was equally effective in controlling furunculosis whether coated on or incorporated into pellets.

Data from the aquarium and raceway studies were analyzed using the 2×2 chi square test. Differences between the treated and control groups were found significant at the 95% confidence limits.

Discussion

Both in vitro and in vivo tests showed that Ro5–0037 was more effective in inhibiting *A. salmonicida* than were either of its components, sulfadimethoxine

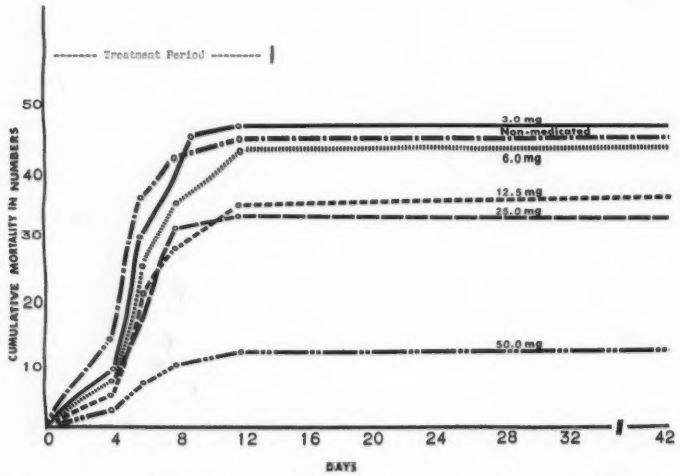

FIG. 3. Comparison of different dosage rates of Ro5–0037 in controlling experimentally-induced furunculosis in rainbow trout (*Salmo gairdneri*). Results from replicate aquaria have been combined.

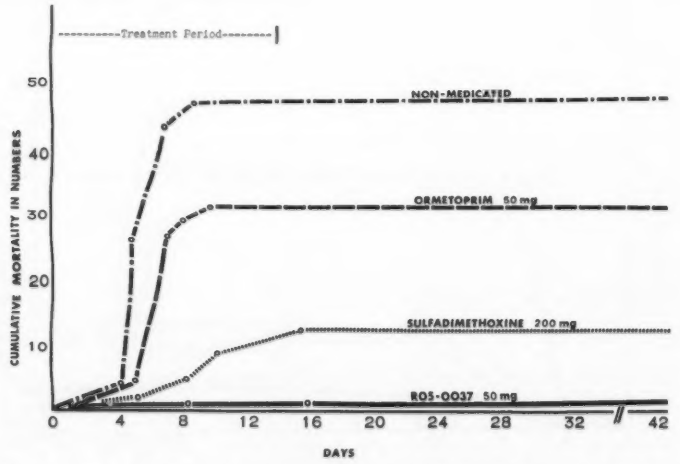

FIG. 4. Effect of 14-day treatment of furunculosis in rainbow trout (*Salmo gairdneri*) with Ro5–0037 and its components, sulfadimethoxine, and ormetoprim. Results from replicate aquaria have been combined.

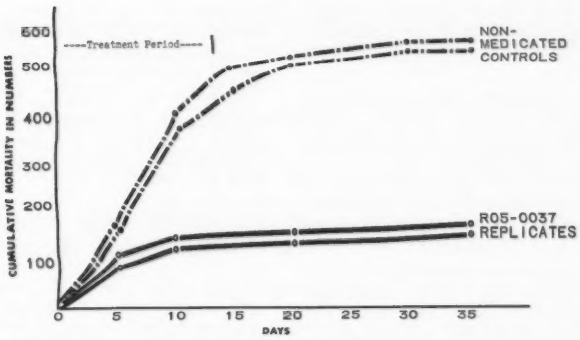

FIG. 5. Effect of 14-day Ro5–0037 treatment (50 mg/kg of trout/day) of furunculosis in 6600 brook trout fingerlings (73/kg) being raised at Tylersville NFH, Lamar, Penn.

or ormetoprim, used alone. The tube dilution sensitivity tests showed Ro5–0037 was more effective than either of its components against both sulfonamide-sensitive and resistant strains of *A. salmonicida*. While all but one of the resistant strains required 500 μg/ml sulfadimethoxine for inhibition of growth after 24-hr incubation, only 0.8–20 μg/ml Ro5–0037 was required to inhibit the same strains. Furthermore, the three strains tested required more passages to become resistant to Ro5–0037 than to sulfadimethoxine or ormetoprim used alone.

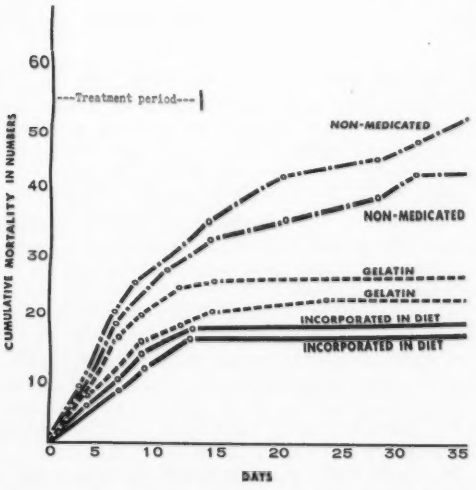

FIG. 6. Effect of 14-day Ro5–0037 (50 mg/kg of trout/day) treatment of furunculosis of 1545 brown trout fingerlings (22/kg) at Fisheries Center, Spearfish, S.D. Ro5–0037 was coated onto pellets using 5% gelatin water or pelleted as part of diet.

The potentiating effect of ormetoprim was further demonstrated by the in vivo studies. The recommended sulfonamide level for furunculosis therapy is 200 mg/kg of trout/day for 14 days; effective control of experimental infections or natural furunculosis outbreaks was achieved with Ro5–0037 at 50 mg/kg of trout/day for 14 days. The addition of ormetoprim increased the effectiveness of sulfadimethoxine. This is in agreement with the findings of Hoffer et al. (1971) who reported that addition of ormetoprim to sulfisoxasole increased the effeciency of that sulfonamide in the treatment of several gram-positive and gram-negative bacterial infections in mice by 1.4 to over 11 times. In the present work ormetoprim alone was not effective in furunculosis control, even at six times the level found in Ro5–0037.

These studies indicate the value of Ro5–0037 in treating furunculosis lies in its effective control of the disease at one-fourth the usual sulfonamide level, its in vitro effectiveness against sulfonamide resistant strains of *A. salmonicida* and also its lack of toxicity to trout. Experiments performed at the EFDL have shown no evidence of gross or histological toxic effects in all viscera, kidney, or muscle when Ro5–0037 was fed at up to 500 mg/kg of trout/day for 14 days.

Acknowledgments

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and furnishing us with data. Mrs M. Landolt, EFDL, and Dr D. Scarpelli, Kansas Medical School, examined tissue sections.

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Behavioral Ecology of Chum Salmon Fry (*Oncorhynchus keta*) in a Small Estuary

J. C. MASON

Department of the Environment
Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C.

MASON, J. C. 1974. Behavioral ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

Chum salmon fry (*Oncorhynchus keta*) in the estuary of a small coastal stream exploited fresh water, estuarine, and marine food chains and, by so doing, were exposed to marked, daily fluctuations in salinity that demanded active selection of fresh water on ebbing tides day and night. The resulting delay in seaward migration and associated behavioral observations are inadequately reflected in published accounts of the life history and behavior of chum fry in natural systems and laboratory situations, and the downstream displacement theory. The biological basis for delayed seaward migration of chum fry merits the attention of fishery researchers and resource managers alike.

MASON, J. C. 1974. Behavioral ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

Des alevins de saumon keta (*Oncorhynchus keta*) habitant l'estuaire d'un petit cours d'eau côtier exploitent les chaînes alimentaires d'eau douce, d'estuaire et d'eau salée, et ce faisant, sont exposés à des fluctuations journalières de salinité qui nécessitent un choix actif de l'eau douce au cours du reflux, tant de jour que de nuit. Le retard qui en résulte pour la migration vers la mer et les observations sur le comportement associé à ce phénomène ne sont pas adéquatement couverts dans les comptes rendus du cycle biologique et du comportement des saumons keta dans la nature et au laboratoire, non plus que dans la théorie de la migration en aval. Les fondements biologiques de la migration retardée vers la mer du saumon keta méritent l'attention tant des scientifiques des pêches que des aménagistes de la ressource.

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FRY of the chum salmon, *Oncorhynchus keta*, have been reported actively feeding in fresh water throughout much of their natural range. Considerable growth may occur while in fresh water since rather large fry (58–82 mm TL) were captured in the Nooksack River, Washington by Tyler and Bevan (1964). Fry exceeding 66 mm were trapped in the Cowichan River, British Columbia, by Sparrow (1968), and Semko (1954) states that chum fry of the Bolshaia River in West Kamchatka attain 53–55 mm by June and July at time of entry into the sea.

Although adult chum salmon commonly migrate 100 miles or more to reach spawning grounds in large river systems, in most coastal streams of British Columbia and Washington spawning extends from intertidal areas upstream a few miles. In short coastal streams chum fry may reach the sea in less than one night (Neave 1955) yet in such systems many fry grow to a considerable size while in fresh water. Apparently, and to varying degrees, chum fry can delay downstream migration and feed vigorously. Some chum fry migrating seaward from the Big Qualicum River exceeded 60 mm FL, the average length of fry increasing as the season progressed (Walker and Lister 1965). Also on Vancouver Island, a few miles northward, chum salmon have occasionally spawned in Lymn Creek within ½ mile of the estuary, yet fry exceeding 70 mm FL have been captured by the author in fresh water as late as June 3.

In addition to illustrating delayed migration seaward from a short coastal stream, chum fry in Lymn Creek were found only in the estuary. Here, their behavior and ecology were influenced by the tidal cycle. This paper reports on the behavior and feeding ecology of chum fry in Lymm Creek as they relate to the estuary and the tidal cycle. These and other appropriate data are considered with reference to the downstream displacement hypothesis proposed for juvenile salmonids by Hoar (1958). Evidence is presented showing that these chum fry were exposed to tide-induced salinity changes subsequently demanding active selection

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of fresh water by marked fry during three consecutive days. Furthermore, these consistent behavioral responses served to delay movement seaward and allow diel exploitation of freshwater, estuarine, and marine food chains prior to entry into the sea.

Study Area and Methods

Chum fry were first observed and captured by seine during low tide at station 4 (Fig. 1) on May 10, 1968. To gain some idea of their general distribution in the estuary, the estuary was seined intensively during low tide on May 13 at five stations (1–5), starting at station 1, at about 364-m intervals. Some 91 m of stream were seined at each station. Captured fry were marked by fin excision and released at point of capture. Three days later on May 16, the stream was seined again on high tide in the early morning at stations 4 through 7 and, in the afternoon on low tide, at stations 1 through 4. Station 4 was seined again on low tide on June 3. The upper estuarial zone was also seined in late May 1969 and 1972 for corroborative data, and the mark and recapture routine was repeated at station 4 in 1972. Captured fry were preserved in 10% formalin for laboratory analysis.

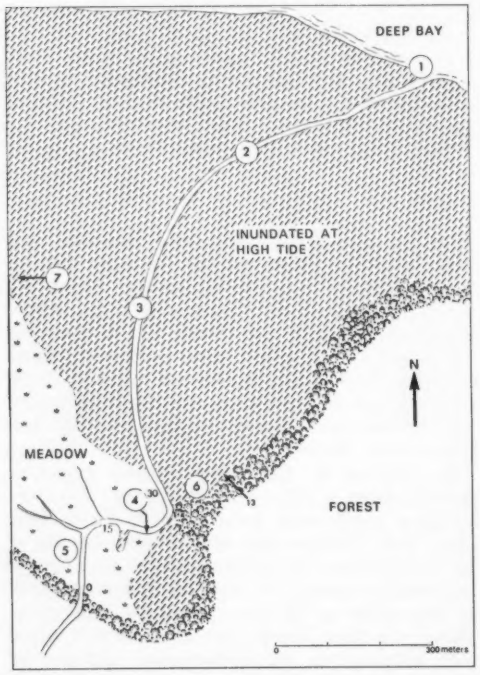

FIG. 1. Lymn Creek as it enters Deep Bay with collection stations referred to in the text. Uncircled numbers refer to 10's of meters downstream from an arbitrarily placed zero point in fresh water.

In the laboratory, preserved fry were examined for marks and catches from each station were subsampled for measurement of fork length. For dietary analysis, size-matched fish were selected from each subsample, reflecting the variation in size within the catches. The entire gut was removed and the contents of the anterior stomach, posterior stomach, and anterior intestine were examined using a dissecting microscope. The relative importance of the various dietary components was assessed by allotting (by eye) a percentage of the volume of each gut section to the various dietary components and by counting the actual number of animals present in each component, including animals reconstructed from chitinous fragments. Close agreement of results obtained by both methods indicated no significant possible source of error attributable to unequal digestion rates for the several categories of food items consumed by the fry.

Both stream drift and benthos were sampled in the estuary on low tide. In addition, zooplankton samples were taken at high tide immediately downstream from the area where chum fry concentrated in fresh water during low tide. The drift and zooplankton samples were sorted to taxa and relative volume assessed by eye. Taxa in the benthos were assessed by settled volume.

Fry behavior was observed closely during low tide and throughout the incoming high tide (afternoon) for 3 days at station 4 from benches placed in the stream.

Results

Fry Distribution and Tidal Cycle

Behavior and distribution of fry were influenced by the tidal cycle. In 1968, nearly 1500 chum fry were captured by seining in various locations in the estuary and, with one exception (station 4), fry were schooled when netted. At low tide, when freshwater flow extended downstream to station 1, schooled fry were abundant at station 1 where the stream emptied into Deep Bay (Fig. 1), unschooled fry were abundant at station 4, but fry were scarce or absent in the intermediate section of stream (Table 1). At high tide, schooled fry were abundant at stations 6 and 7 but fry were absent from station 4 despite intensive seining.

During low tide at station 4, the abundant unschooled fry occupied a specific glide about 27.3 m long and just downstream from a wide, shallow riffle. During low tides, ebbing below 3.35 m, water depth over the gravel bottom of the glide varied between 10 and 15 cm, and current velocity exceeded 0.40 m/sec. During high tide, flooding above 3.66 m, depth increased to about 0.61 m, and current velocity slackened to <0.07 m/sec. For some 46 m below the glide, the stream deepened to 0.61 m over a silty–mud bottom, contained an extensive bed of sea lettuce (*Enteromorpha linza*), and became 1.22–1.52 m deep during high tide. The gradient increased seaward

of this stream section, the stream flowing shallow and fast over a rubble–gravel bottom until emptying into Deep Bay. No fry were ever captured or observed upstream from station 4 on either high or ow tidal phases despite intensive seining of suitable habitat during May and June 1968, 1969, and 1972.

The fry were observed simultaneously during the day from two locations at station 4, on both phases of the tide. During low tide chum fry were scattered over the stream bed, maintaining consistent positions 10 or more centimeters apart, and feeding vigorously on midwater and surface drift, and on the benthos. Both intra- and interspecific interactions were seen, the latter with coho fry (*O. kisutch*) which were smaller, the smaller fish being chased off.

On the rising tide, feeding chum and coho fry, and gammarid amphipods, moved slowly upstream as current velocity decreased and depth increased. When the rise in tide sufficed to cancel perceptible stream current velocity, only chum fry were seen wandering about in many directions. Further rise in the tide produced a low velocity, upstream current not exceeding about 0.07 m/sec. Only chum fry swam actively into the tidal current and some proceeded slowly seaward from station 4. At the peak of flood tide, the salinity at the seaward end of station 4 was 26.9‰; that of station 7, a shallow area of beach subject to local surface water drainage, was 25.5‰.

Temperatures of the stream and of adjacent surface waters of Georgia Strait during May were similar (8–12 C), one not exceeding or falling below the other by more than 2 C.

Recapture of Marked Fry

In 1968, some 21% of the fry captured, marked, and released at stations 3 and 4 (upper intertidal) in fresh water during low tide were recaptured 3 days later at several locations in the estuary on both low and high tides (Table 1). No fry marked and released at station 1 (lower intertidal) were recaptured, probably due to the strong lateral transport of Georgia Strait water. These fry were excluded from the mark recapture calculations which are intended to illustrate downstream movements of marked fry. Half of the recaptured fry were taken in Georgia Strait water >26‰ salinity and were schooled when netted but the remainder were unschooled fry retaken in fresh water at station 4. In relative terms, recaptured fry were more than four times more frequent in the catch at station 4 than at the other three stations, suggesting that the original population (May 13) at station 4 had a discrete identity despite a high rate of loss due to downstream movement.

In 1972, 17% of the fry captured, marked, and released at station 4 were recaptured there 3 days subsequently. In both years, the recaptured fry included individuals as small as 36–37 mm FL.

Size Distribution in Fry Catches–Recruitment and Growth Effects

In 1968, considerable variation in size of fry in the various catches (Fig. 2) suggests that catches were not drawn from a homogeneously distributed population ($F_{5,\ 457} = 33.8$). Size differences are attributed to growth and recruitment, the latter of emergent fry or fry recruited from adjacent spawning systems. Average size of fry captured at station 4 at 3-day intervals differed significantly from each other ($P < 0.05$), average size increasing from May 10 to 13, then decreasing sharply on May 16 (Table 2). Fry <40 mm were absent from the catches prior to May 16. On that date, many fry between 33–40 mm were taken, indicating recruitment of newly emerged fry (Fig. 2).

When tested by Student's "*t*" test, the average size of fry in the various catches made in 1968 differed significantly between all catches except for one pair: station 4, low, and station 6, high, on May 16 (Table 2). These latter catches were made about 61.0 m apart on successive tides and may reflect the seaward movement of fry from station 4. Average size of fry captured at station 4 in 1970 did not differ significantly between dates.

Comparative Dietaries and their Food Sources

Although amphipods, copepods, and insects composed more than 95% of the organisms consumed at all sampling stations (Table 3), the dietaries of fry captured in fresh water at station 4 differed markedly from those of fry taken at stations 1, 6, and 7 downstream in the estuary. Dietaries at stations 1, 6, and 7 were quite similar. Dietary differences at station 4 entailed both disproportionate consumption and temporal changes in consumption of commonly eaten food items and these differences are considered below by station.

Station 4, low tide, fresh water — Fry fed chiefly (71–88% by volume) on amphipods, primarily on the gammarids, *Anisogammarus confervicolus* Stimpson and *Paramoera columbiana* Bousefield, and secondarily on *Corophium spinicorne* Stimpson. There was a shift in importance of various dietary components proceeding posteriorly along the gut, suggesting that the diet changed in time, and therefore with the tidal cycle. For example, copepods,

chiefly harpacticoids, never composed more than 3% of the anterior stomach contents but made up 19–68% of the anterior intestine contents while amphipods decreased correspondingly. Posterior stomach contents represented an intermediate condition. The diet of recaptured fry (marked) closely matched that of other fry.

One may tentatively conclude from these dietary data that chum fry captured at station 4 in fresh water were exposed to saline water on the previous high tide, and that some fry (those recaptured in fresh water) had been exposed to consecutive tidal phases and were feeding successively on fresh water, estuarine, and marine food chains.

Station 1, 6, and 7, both tides, salt water — Fry showed a common feeding pattern but dissimilar to that of fry from station 4 at low tide, harpacticoid copepods rather than amphipods predominating in their diet (78–92%). The same amphipods were consumed but their importance was much reduced (1–17%). The definite shift in species composition along the gut observed in fry from station 4 at low tide was not seen in reverse order in fry from these saltwater stations. Rather, the relative importance of dietary components remained consistent throughout the gut, suggesting no exposure to fresh water during the previous tide.

Stream benthos and drift in relation to diet of chum fry — The diet of chum fry at station 4 was more influenced by the composition of the drift than of the benthos. Amphipods and isopods, mainly the latter, made up 89–97% of the benthos in the upper estuary (Table 4). The isopod, *Exosphaeroma oregonensis* (Dana), composed some

TABLE 1. Catches and recaptures of chum salmon fry (*Oncorhynchus keta*) in Lymn Creek estuary during May.

Date, tide, and station	No. of fry caught	Marks applied or recaptured	% of catch as recaptured marks	% of marks UC and V recaptured
May 10, 1968				
Low-4	38	(Initial seining) Catch retained		
May 13, 1968				
Low-1	66	Lower caudal lobe — LC	Marked and released at station where captured	
2	0			
3	9	Upper caudal lobe — UC		
4	131	Left or right pelvic — V		
5	0			
May 16, 1968				
High-1 to 3		These stations on the flooded tidal flat not sampled		
4	0			
5	0			
6	328	6 V	1.8	4.3
7	115	2 V	1.7	1.4
Low-1	702	8 V	1.1	5.7
2	0			
3	0			
4	180	14 (1 UC, 13 V)	7.8	10.0
5	0			
May 16, 1968 Total	1325	30		21.4
May 26, 1972				
Low-4	200	Upper caudal lobe — UC	Marked and released where captured	
May 29, 1972				
Low-4	556	34 UC	6.1	17.0

57–88% of the benthos but was present in the drift to a minor degree (Table 5), rarely being consumed by the fry. Conversely, gammarid amphipods dominated the drift, forming the bulk of the fry's diet, but constituted two to five times less bulk in the benthos at station 4 than did isopods.

Oligochaetes were taken in both drift and benthos throughout the upper estuary but were present only in the stomachs of fry from station 4. The tube-dwelling amphipod, *C. spinicorne*, was not taken in the drift and was present in the benthos only in the vicinity of station 4 (Table 4). This amphipod was commonly eaten (50–80% incidence) by fry at station 4, uncommonly eaten (<25%) by fry

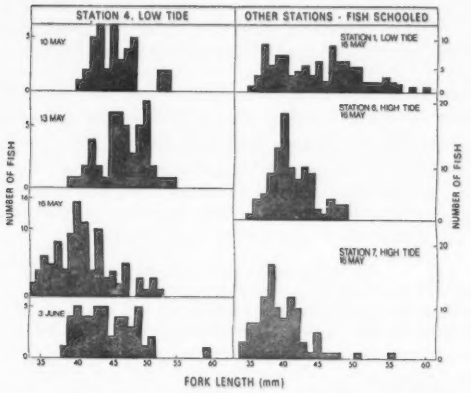

FIG. 2. Length-frequency histograms for catches of chum fry at Lymn Creek, 1968.

TABLE 2. Size of chum fry collected in Lymn Creek.

Date, station, and tide	No. of fry measured	Fork length (*mm*) $\bar{X}$	SE	Range
May 10, 1968				
Sta. 4	38	45.5	0.54	40–53
May 13, 1968				
Sta. 4	50	47.0	0.49	39–54
May 16, 1968				
Sta. 1	100	45.2	0.61	35–61
4	100	41.0	0.44	35–52
6[a]	100	41.4	0.32	35–49
7[a]	100	39.8	0.37	34–56
June 3, 1968				
Sta. 4	50	44.7	0.58	38–59
May 27, 29, 1969				
Sta. 4	21	49.5	0.41	38–55
May 26, 1972				
Sta. 4	148	42.8	0.58	35–70
May 29, 1972				
Sta. 4	552	42.0	0.21	33–70

[a]High tide; low tide at other stations.

TABLE 3. Diet of chum fry in Lymn Creek estuary, 1968, expressed as mean percentages of the total food items in three regions of the gut. Second value indicates standard error; a — amphipods, c — copepods, i — insects, composed more than 95% of the organisms consumed.

			Anterior stomach			Posterior stomach			Anterior intestine		
Date	Station	Tide	a	c	i	a	c	i	a	c	i
May 10	4	low	90.3	1.5	8.2	88.6	5.9	5.5	67.5	30.3	2.2
			2.8	0.6	2.9	2.6	1.7	2.7	6.3	6.2	2.1
May 13	4	low	73.9	5.3	20.8	67.6	7.9	24.5	31.5	20.6	47.9
			6.8	1.9	5.1	7.4	3.0	7.2	10.2	8.0	10.8
May 16	4	low	93.6	2.7	3.7	78.0	15.5	6.5	32.0	62.2	5.8
			1.6	1.3	1.1	5.6	6.1	1.2	8.4	8.8	3.1
May 16	4	low*	90.8	2.0	7.2	74.4	18.6	7.0	27.1	67.6	5.3
			2.2	0.7	1.9	6.6	5.9	1.5	6.8	6.4	2.8
May 16	1	low	2.9	90.4	6.7	10.5	86.0	3.5	9.5	89.8	0.7
			1.7	6.3	5.9	4.2	5.3	3.1	4.8	4.8	0.4
May 16	6	high	9.0	87.4	3.6	16.6	78.2	5.2	17.2	80.3	2.5
			2.9	3.4	1.2	4.6	4.8	3.4	8.0	9.7	2.5
May 16	7	high	0.2	92.4	7.4	8.5	91.5	0	0	100.0	0
			0.2	2.3	4.4	4.6	4.6	0	0	0	0

*Recaptured fry that were marked and released on May 13.

at stations 6 and 7, and was absent in the digestive tracts of fry at station 1. The cyprid stage of the barnacle was present only in the posterior stomach and intestine of station 4 fry but occurred commonly throughout the tracts of fry from other stations, and was taken in the drift downstream from station 4 on high tide (Table 5). Chum fry did not eat the nauplius stage of the barnacle despite its abundance in high-tide water.

Calanoid copepods, mainly *Calanus plumchrus*, fifth copepodid stage, were abundant in high-tide water but absent in the upper estuary. Although calanoids were commonly eaten (50–75% incidence) by fry from stations 6 and 7 on high tide, more than 75% of the copepods consumed there were harpacticoids. Although the fry captured at station 4 in fresh water had been feeding primarily on gammarid amphipods, the posterior stomachs of three fry (5% incidence) contained small numbers of *C. plumchrus*, fifth copepodid stage. These calanoids were most likely eaten during the previous high tide.

Insects, mainly Diptera, were very commonly consumed (92–100% incidence) by fry from all stations except station 1 where their incidence dropped to 31%. The relative composition of insect forms was not uniform in the various dietaries. For example, fry ate three to five times as many larvae and pupae as adult insects, mainly Diptera, at station 4. At the other stations downstream, fry consumed as many adult insects as larvae and pupae.

Comparative dietaries of chum and coho fry at station 4 — Large numbers of coho fry moving downstream from spawning areas reach the estuary by the end of May. Catches of both chum and coho fry were made at station 4; the chums averaging 48.6 mm FL (38–55 mm), the coho averaging 38.3 mm FL (36–43 mm). Chum fry occupied areas offshore in higher water velocities than did coho which tended to remain inshore but considerable distributional overlap was observed (see previous comments on interspecific interaction).

Despite the general similarity in their diets, dissimilarities occurred possibly reflecting different responses to the tidal cycle. Dietary comparisons are made below for major food items.

TABLE 4. Benthic invertebrates taken on low tide in Lynn Creek estuary, expressed as a percentage of the total volume of organisms at each station. Stations refer to distance in meters downstream from an arbitrarily placed zero in fresh water (Fig. 1).

Taxon	Sampling station				
	30	107	137	191	244
Mollusca	3.5	0.6	<0.1	<0.1	–
Oligochaeta	1.2	0.2	2.2	0.5	1.1
Polychaeta	–	<0.1	0.5	0.2	0.3
Hydracarina	<0.1	–	–	–	–
Isopoda					
E. oregonensis	<0.1	87.8	81.0	76.6	57.3
Amphipoda					
A. confervicolus } [a]	<0.1	9.2	11.6	14.5	31.2
P. columbiana } [a]					
C. spinicorne	–	–	–	<0.1	9.8
Copepoda					
Harpacticoidea	–	<0.1	<0.1	–	–
Insecta					
Coleoptera	–	–	<0.1	–	–
Ephemeroptera	52.0	0.1	<0.1	–	–
Plecoptera	2.9	–	–	–	–
Trichoptera	11.6	0.3	1.2	–	0.3
Diptera					
larva	16.8	0.5	0.5	0.1	0.1
pupae	8.7	0.1	–	–	–
misc.	3.5	0.1	0.8	–	–
total	29.0	0.7	1.3	0.1	0.1
Total volume (cm^3)	1.73	17.42	8.65	12.40	12.20

[a] Predominantly *A. confervicolus*.

A. confervicolus — present throughout the tracts of all fry of both species and constituting 80% of both diets.

C. spinicorne — very common throughout the tracts of chums and stomachs of coho but uncommon in intestines of coho. Twice as important in coho stomachs (20%) as in chum stomachs (10%<).

E. oregonensis — absent in chums, uncommon but throughout the tracts of coho, constituting 2% of the diet.

Annelida — present in both fry but only in the anterior stomach. Uncommon in coho but very common in chum, constituting 10% of their diet.

Harpacticoida — in chums, uncommon in stomachs but very common in intestines (7%). Uncommon throughout the tracts of coho and 1%< of volume.

Cirripedia cyprid — absent in coho, present only in posterior stomach and intestines of chums.

Discussion and Conclusions

Considerable numbers of chum fry apparently remained but temporarily at station 4 on the flooding tide and moved downsteram somewhat at the height of flood and back upstream during the ebbing tide. Direct observation of their behavior at station 4 during flooding and ebbing tides supports this conclusion. Similarly, dietary shifts in time, obviously related to the tidal cycle, are suggested from analysis of their digestive tracts. Further support for this conclusion is lent by the high recovery rate of marked chum fry at station 4 during low tide (10% and 17%), 3 days subsequent to their release, their contribution to the catch at this station (6–8%), and the absence of fry upstream from station 4 on either high or low phases of the tide and at station 4 at high tide. Apparently, these fry underwent daily salinity transitions from 0 to 27‰ and back again to fresh water and, in the case of recaptured fry, for at least 3 consecutive days that included low tides at night.

In contrast to chum fry during the day, coho fry at station 4 were not observed wandering or moving seaward at the height of flood tide. Changes in dietary components along the gut in relation to distribution of these components in the benthos of the upper estuary suggest that coho at station 4 moved upstream and stayed in fresh water during the flood tide. For example, when captured at station 4 on low tide in fresh water, harpacticoid

TABLE 5. Drifting invertebrates captured by stationary nets fished for 30–50 min during low tide in Lymn Creek. Values in brackets are percentages of drift volume. Stations refer to distance in meters downstream from an arbitrarily placed zero in fresh water (Fig. 1).

Taxon	Sampling station							
	91	137	213	244	259	305	305[a]	396[a]
Oligochaeta	4(15)	–	–	–	–	–	8(3)	–
Hydracarina	3(35)	1(1)	–	–	1(<1)	–	–	–
Isopoda								
E. oregonensis	–	3(23)	2(25)	–	–	–	–	–
Amphipoda								
A. confervicolus, *P. columbiana*[b]	4(15)	18(75)	4(75)	16(100)	4(100)	2(100)	38(43)	13(8)
C. spinicorne	–	–	–	–	–	–	2(3)	7(3)
Cirripedia								
nauplius	–	–	–	–	–	–	–	125(3)
cyprid	–	–	–	–	–	–	4(1)	2(<1)
Copepoda								
Harpacticoidea	–	–	–	1(<1)	–	–	16(20)	6(2)
Calanoidea	–	–	–	–	–	–	36(30)	300(81)
Ostracoda	5(10)	4(2)	2(1)	–	–	–	–	–
Crab zoea	–	–	–	–	–	–	–	5(2)
Insecta								
Diptera larva	6(12)	1(<1)	–	–	–	–	–	–
adult	1(12)	–	–	–	–	–	–	–

[a]High tide.
[b]predominantly *A. confervicolus*.

copepods were uncommon throughout their tracts but were uncommon only in the anterior stomachs of chum fry. The amphipod, *C. spinicorne*, although twice as important in coho stomachs as in chum stomachs, was uncommon in coho intestines but very common throughout the tracts of chum fry. This amphipod and harpacticoid copepods were absent or rare in the drift and benthos upstream from station 4 (Tables 4 and 5).

The present results are at variance with several accounts of the behavior and life history of chum fry in natural freshwater environments. Hoar (1951) stated that chum fry almost invariably maintain position in schools in fast current during the day; Lymn Creek fry were solitary and aggressive but behaved similarly otherwise during the day while in fresh water while only those fry captured in salt water were schooled. Neave (1955) pointed out that pink and chum fry are rarely seen during the day but Lymn Creek fry in considerable numbers were readily observable from distances less than 1 m in shallow water. Aggression in schools of chum fry was occasionally observed in the laboratory by Hoar (1954) and considered to be a displacement reaction (behavior out of functional context — Armstrong 1950) but unschooled chums in Lymn Creek interacted aggressively with other chums and with coho fry, chasing both away from obviously preferred feeding stations in the stream bed.

In the laboratory, both pink (*O. gorbuscha*) and chum fry (the latter of similar size to chums in Lymn Creek) were found to make a rapid and intensive preferential response to either isotonic or hypertonic sea water (Houston 1957), although using similar techniques, McInerney (1964) reported temporal changes in salinity preference of chum fry from avoidance in early May to a modal preference for 5‰ in late June, 11‰ in mid-July, and 16‰ by mid-August. Reduced locomotor activity of chum fry has been reported to follow sudden transitions in salinity (Shepard 1948; Houston 1957), the latter author postulating that lowered activity on first entry into sea water may enhance seaward movement through passive displacement. Chum fry in Lymn Creek moved into and out of high salinity water, returned to fresh water, and apparently fed while in both media. Houston also noted that chum fry displayed a strong preference for original compartments in his laboratory apparatus, and commented on their definite preference for recognized areas. This ability may, in part, account for their return to station 4 in Lymn Creek on ebbing tides.

Return to preferred areas at night (station 4) calls for recognition of nonvisual directive cues in the natural environment. Other juvenile salmonids do exploit such cues. McDonald (1960) found that up to 40% of sockeye fry introduced into wooden troughs placed in the stream bed failed to pass downstream into traps during the night but maintained position in the upstream end of the trough, oriented into the current, throughout the night. Similarly, coho fry in volitional residence in large stream aquaria (Mason 1969) failed to show a lessened tendency to inhabit fast water and be quiet at night, responses that would appear to reduce downstream displacement (Hoar 1958). Rather, density of fry in riffles doubled during the night and involved a high degree of site selection by some fry. The latter was discovered by using fixed position flash photography and identifying individual fry from their parr marks, at hourly intervals throughout the night. Since the fry bolted into downstream pools following each electronic flash, their return to specific positions in the riffles demanded recognition of nonvisual spatial reference features, possibly specific patterns of turbulence or associated point sources of sound.

Chum fry in Lymn Creek and other systems referred to clearly delay seaward migration and undergo considerable growth while in fresh water. This delay is not accounted for in the general mechanism underlying downstream movement to sea of juvenile salmonids proposed by Hoar (1958). For chum fry in Lymn Creek, disoriented movement during hours of darkness would have resulted in rapid displacement donwstream to sea on the ebbing tide. Seeking fast water at night (Hoar 1958) would result in their passing to sea on the ebbing tide within a few minutes. Active resistance to displacement downstream involving effective positive rheotaxis during darkness and/or following exposure to high salinities must account for my recapture of marked fry in fresh water following nightly high and low tides which alternatively flushed station 4 with sea water and fresh water.

Evidently internal and external environmental factors which motivate downstream migration and movement into the sea cannot be fully explained on the basis of rising water temperature (Hoar 1951), or flow (McDonald 1960), and thyroid stimulation (Hoar 1953) associated with osmoregulatory stress (Hoar and Bell 1950; Eales 1963). In Lymn Creek estuary, temperature differences between the freshwater stream and tidal water (approximately 2 C in either direction) exceeded the threshold detection level by some 40 times (Bardach and Bjorklund 1957). Simultaneous exposure to temperature and salinity changes in estuaries probably have an interactive influence on motivation for seaward movement, perhaps appropriate to

fish recaptured downstream from sites of original capture, marking, and release.

The chum fry in Lymn Creek estuary remain of uncertain origin since no spawning runs were observed during the period 1967–71. No adult chums passed a weir operated at the head of tide during 1971 so fry captured in May, 1972 originated from intertidal spawning of unknown magnitude or were recruited via the sea from other rivers.

Delayed migration seaward, including extended residence in estuaries, or temporary return to estuaries following transport to sea, may be related to availability of suitable food resources in rivers and estuaries. The abundant amphipod populations of the upper estuary in Lymn Creek formed the main diet of resident chum and coho fry. Gammarid amphipods also formed the bulk of the diet of chum fry collected in late spring during low tide from the upper intertidal zone of six streams nearby. Fry from these streams commonly showed an anterior–posteriors shift in composition of prey species along the gut: amphipods–chironomids; but one in contrast to the amphipod–harpacticoid copepod shift found in Lymn Creek chum fry and this is interpreted as signifying recent recruitment to the estuary from upstream areas. These chum fry were considerably smaller than Lymn Creek fish, ranging from 34 to 45 mm FL, and fry exceeding 40 mm FL were found only in a small stream adjacent to Lymn Creek which also flows into Deep Bay.

Although one might expect a higher relative energy cost for swimming and feeding in rapid, freshwater flow than in the sea, delayed seaward movement may be of adaptive value to chum fry resident in some rivers and estuaries from a bioenergetic standpoint. For comparable gut volumes, fry in the upper estuary of Lymn Creek consumed somewhat less than half the number of food items (gammarid amphipods) than those fry feeding on harpacticoid copepods in the adjacent sea.

The trophic reliance of the latter fry on harpacticoids corroborates the recent report of Kacznski et al. (1973) on intertidal chum and pink fry in Puget Sound. Our findings suggest rather specialized feeding behaviors during this life history phase that are possibly related to patterns of localized turbulence in marine shallows.

Chum fry may assume residence in the intertidal zone of Lymn Creek for 30 days or more, the average time falling between 1–2 weeks, based on application of the estimated instantaneous growth rates derived from pink salmon fry by LeBrasseur and Parker (1964). Extended time in residence may entail disadvantages. Although the marine survival rate may be several times higher for resident fry, mortality in the intertidal zone may offset any potential population advantage.

The present observations and recent literature indicate that young chum salmon may show complex patterns of transition from fresh to sea water in some river systems. These complexities (ecotypic diversities) are not adequately reflected in the commonly accepted account of the early life history and behavior of chum salmon, or in the downstream displacement hypothesis. Both evidently require reexamination with a view to improving their relevance.

Finally, the data presented here strongly suggest that where man-made changes lead to deteriorating estuaries of river systems in which delayed seaward migration occurs chiefly in the lower reaches, remedial programs must consider this broader concept of behavior.

Acknowledgments

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OBITUARY

Archibald Gowanloch Huntsman

1883–1973

Dr Archibald Gowanloch Huntsman (familiarly known as "A.G.") died suddenly on August 8, 1973, and Canada lost an outstanding marine biologist of this century. True to his tradition, he was active in investigation and criticism to the end. Though in his 90th yr, and officially retired for two decades, his interest in how to produce more Atlantic salmon for the anglers had kept him busy in body and mind. Nor were his interests narrow; they extended not only to a great many biological subjects but also to religion and philosophy.

Born in the small community of Tintern, (near St. Catharines) Ontario, on November 23, 1883, he received his Bachelor of Arts at the University of Toronto in 1905 and his Bachelor of Medicine (subsequently converted to MD) 2 yr later, but he never practised medicine. Instead, he was caught by the intriguing problems of how the sea produces fish and fisheries. Throughout his career from 1904 to 1973 he was identified with the development of the Fisheries Research Board of Canada and its forerunners — the Board of Management of the Marine Biological Station (1898–1912) and the Biological Board of Canada (1912–38). Indeed, such was his energy and force of character that he dominated the Board scene for many years, and it may well be doubted whether the Board would have survived its early critical years without him.

Although he remained associated with the University of Toronto for 50 yr, he was director of the Board's St. Andrews Biological Station from 1911 to 1933, concurrently director of the Halifax Laboratory from 1924 to 1929, and Editor of the Board's publications from 1933 to 1949.

His interests were varied, from the systematic study of tunicates through the life histories of many commercial species to the oceanographic mechanisms responsible for marine productivity and fisheries. He was able to infect others with his insatiable curiosity, and for many years combined fisheries research with postgraduate teaching at Toronto. A majority of Canada's fisheries biologists educated in the 1920s and 1930s were, at some stage, his students. One of these, Dr J. L. Hart, has written an excellent "Appreciation," which appeared as the introductory paper in a special issue of the Board's Journal (Vol. 22, No. 2, 1965) published in Dr Huntsman's honor. Among the many honors and important appointments listed in the "Appreciation," the following are noted here: he was a Vice-President of the British Association for the Advancement of Science in 1924; President of Section V (Biological Sciences) of the Royal Society of Canada, 1924–25, and President of the Society in 1937–38; President of the American Fisheries Society in 1936–37; and recipient of the Flavelle Medal of the Royal Society of Canada.

For those of us who knew him for many interesting years and admired his energy, his stimulating argument and criticism as a self-styled "gadfly," his breadth of interest, and his indomitable spirit, he leaves a gap that will never be filled in a like manner.

A. W. H. Needler

NOTES

Preliminary Feeding Trials Using Artificial Diets to Study the Nutritional Requirements of Oysters (*Crassostrea virginica*)

J. D. CASTELL AND D. J. TRIDER

Department of the Environment
Fisheries and Marine Service, Halifax Laboratory, Halifax, N.S. B3J 2R3.

CASTELL, J. D., AND D. J. TRIDER. 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Board Can. 31: 95–99.

Oysters (*Crassostrea virginica*) showed positive growth response when fed artificial diets of known nutrient composition; differences in growth rate, meat weights, and glycogen content corresponded with differences in dietary composition. High levels of dietary carbohydrate resulted in greater oyster glycogen production. The type and level of lipid in the diet was also important. Cod liver oil was of higher nutritional value for the oysters than corn oil; increasing the dietary lipid from 5 to 18% of the dry weight, resulted in greater meat production and earlier total weight gains considered to be associated with earlier gonadal ripening.

Oysters held in Malpeque Bay in floating trays grew 10 times faster than those held in tanks of continuously flowing filtered sea water and fed artificial diets.

CASTELL, J. D., AND D. J. TRIDER. 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Board Can. 31: 95–99.

Des régimes artificiels de composition connue et contrôlée avec précision provoquent, chez l'huître (*Crassostrea virginica*), une réponse positive de croissance. Des différences dans le rythme de croissance, le poids des chairs et le contenu en glycogène des huîtres correspondent bien à des différences dans la composition du régime. Les hauts niveaux d'hydrate de carbone dans le régime, qui résultent en une forte production de glycogène chez l'huître et qu'on considérait jadis comme optimum, semblent maintenant excessifs quand on considère d'autres facteurs tels le rythme de croissance et la production de chair.

L'huile de foie de morue a une valeur nutritive plus grande que l'huile de maïs. Ceci peut être associé aux exigences de l'huître en acides gras essentiels (EFA). Une augmentation des lipides dans le régime, de 5 à 18% du poids sec, a pour effet de hâter la maturation sexuelle et de produire plus de chair, tant avec l'huile de foie de morue qu'avec l'huile de maïs.

Des huîtres maintenues sur des plateaux flottants dans la baie Malpèque ont un rythme de croissance 10 fois supérieur à celui d'huîtres maintenues en eau de mer filtrée et nourries de régimes artificiels.

Received September 12, 1972

ALTHOUGH oysters have been cultured for many centuries, little is known of their nutritional requirements. Further advances in oyster culture necessitates an accurate knowledge of these requirements. Biologists and ecologists need this data for assessing the suitability of natural feeds for oyster production. Laboratory workers would like to prepare easily stored, artificial diets, thus eliminating the need to grow large quantities of algae continuously, and making genetic studies and laboratory rearing of oysters much easier.

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A number of studies (Davis and Guillard 1958; Loosanoff and Davis 1963; Carefoot 1967; and Walne 1970) in which cultures of pure and mixed strains of algae were fed to oysters and other molluscs, demonstrated which strains resulted in the fastest growth rates. Attempts to correlate differences in oyster growth with composition of algal species, however, have met with little success (Cowey and Corner 1966; and Walne 1970a). Others placing emphasis on carbohydrates (Haven 1965; Gillespie et al. 1964, 1966; Sayce and Tufts 1967; and Dunatham et al. 1969) fed single components, such as glucose or starch, and various mixtures, such as crab meal

and cereal meals, to oysters and in many cases observed positive growth response. These preliminary studies have provided much valuable information, but a complete picture of the oyster's nutritional requirements can only be gained by using artificial diets, in which each individual nutrient can be independently varied. This paper reports the first attempt at maintaining oysters on this type of artificial diet. Preliminary comparisons were made between two types of lipid, between two levels of lipid and carbohydrate, and between artificial and natural diets.

Materials and methods — A total of 1500 oysters (*Crassostrea virginica*), (average weight 1.38 ± 0.41 g SD) were obtained June 14, 1971, from floating trays held in Malpeque Bay, Prince Edward Island. The oysters were cleaned, sorted randomly into 15 groups of 100 each, and individually weighed. Five groups were placed in separate 0.5 × 0.5 m fiberglass-coated wooden trays, with plastic-coated wiremesh bottoms. These trays of oysters were anchored at various locations in Malpeque Bay where they remained about 5 cm below the water surface and served as natural diet controls.

The remaining 10 groups were held ashore in special trays equipped with baffles, to ensure that water and suspended food passed over each individual. Each tray was supplied with a continuous flow of approximately 110 liters/hr filtered sea water at ambient temperatures. The filters removed particles of 5 μ or larger.

Diets — Each of the four artificial diets was fed to duplicate groups of 100 oysters. Two groups were held as unfed controls. Ingredients listed in Table 1 were mixed thoroughly and stored at approximately –10 C until used. Food was prepared by suspending dry diet in distilled water (0.125% suspension). The diet was kept in suspension by use of Teflon®-coated stirrers and fed by a variable speed peristaltic pump at the rate of 65 ml/hr. Each tray received 500 ml suspension containing 0.625 g of diet, or approximately 0.71 mg of dry diet per liter of sea water for 8 hr each day.

The diets were mixtures of dissolved water-soluble nutrients and suspended particles ranging in size from less than 1 to about 50 μ in diameter. Over 90% of the particles were in the 5–15 μ diameter size range.

Sample analysis — Samples of 10 oysters were selected randomly from each tray at 2-week intervals. At the end of the 12-week study, the remaining 50 oysters were removed from each tray for analysis. The shells were dried and individual live weights recorded. The modified condition index, MCI, (Katansky et al. 1967) was determined for pooled samples of five oysters. Each glycogen determination was made with pooled samples of meats from five oysters. The meats were homogenized in two volumes of 30%, KOH the glycogen was precipitated with ethanol (Good et al. 1933), and the concentration determined by the phenol-sulphuric acid procedure of Montgomery (1957).

TABLE 1. Experimental diets used in oyster nutrition study.

Dietary component[a]	Percent dry wt of diets			
	1	2	3	4
Corn starch	60	20	60	20
Corn oil	5	18	–	–
Cod liver oil	–	–	5	18
Casein (vitamin free)	15	25	15	25
Alpha-cellulose	14.8	31.8	14.8	31.8
Vitamin mix[b]	2	2	2	2
Mineral mix[c]	2	2	2	2
Choline chloride (70%)	1	1	1	1
Vitamin E (250 IU/g)	0.2	0.2	0.2	0.2
Approx cal/g	3.45	3.42	3.45	3.42

[a]All ingredients obtained from Nutritional Biochemical Corporation.

[b]The vitamin mixture was composed of the following, given as percent of mix: thiamine (HCl) 0.32, riboflavin 0.72, niacinamide 2.56, biotin 0.008, Ca-pantothenate 1.44, pyridoxine (HCl) 0.24, folic acid 0.096, menadione 0.08, cobalamine 0.2667, *i*-inositol (meso) 12.50, ascorbic acid 6.00, *p*-aminobenzoic acid 2.00, vitamin D_2 (850,000 USP-g) 0.0245, BHA 0.075, BHT 0.075, vitamin A (500,000 IU/g) 0.50, Celite 73.2343.

[c]Modified Bernhart-Tomarelli salt mix, Nutritional Biochemical Corp.

At the end of the experiment three samples of five randomly selected oysters from each of the floating trays, and two samples of five from each of the shore held trays, were taken for determination of dry meat weight. The pooled meats from each sample were drained for 1 min on nylon mesh, and the dry weight determined (24 hr at 110 C).

Results — The MCI values fluctuated widely over the test period for both sets of oysters. Figure 1 shows the mean MCI for the floating tray oysters. The MCI for oysters fed artificial diets fluctuated so much that it was difficult to draw conclusions about the food value of the diets, based on any single sampling period. When the MCI values for each treatment were averaged for the entire experimental period (Table 2), however, significant differences were demonstrated. Analysis of variance of these data gave an *F*-ratio of 12.48 on 5 and 299 df; thus, showing with 99% confidence, that dietary differences were significant. Both diets containing cod liver oil resulted in oysters with higher MCI values than those with corn oil. When the dietary lipid was cod liver oil, increasing the starch content from 20 to 60% resulted in significantly increased MCI values, however, when corn oil was used, varying the dietary carbohydrate had no significant effect on MCI values.

Figure 2A, B illustrates the mean percent gain in live weight for each sample taken from the natural and

experimental diets, respectively. The floating tray oysters grew 10 times faster than those on the artificial diets. Oysters sampled from dietary treatments resulting in peaks in the curves at the 6th and 10th weeks (Fig. 2B)

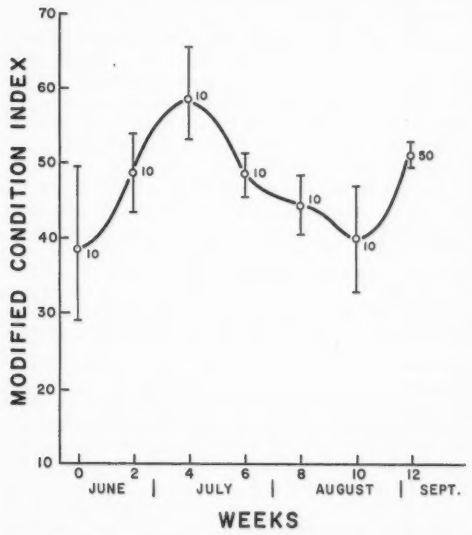

FIG. 1. Average MCI values for natural-fed floating tray oysters. Vertical bars indicate two standard errors, numbers by points indicate number of determinations on pooled lots of five oysters.

TABLE 2. Modified condition index (MCI). Average of all determinations for each treatment over the 12-week feeding period ±2 SE.

Treatment	Avg MCI	No determinations
Floating tray	49.6±1.2	100
Diet 1	43.8±3.1	40
Diet 2	44.4±2.8	40
Diet 3	48.2±3.5	40
Diet 4	46.1±2.8	40
Unfed control	35.5±2.9	40

Least significant difference (95% confidence limits) = 1.7

had enlarged, ripened gonads. Analysis of variance indicated that gains for diets 2 and 4 (18% lipid) were significantly higher than for diets 1 and 3 (5% lipid) with 86% confidence (*F*-ratio of 3.29) at the 6th week sampling, and that for diets 3 and 4 (cod liver oil) gains were significantly higher than diets 1 and 2 (corn oil) with 98.6% confidence (*F*-ratio of 5.01) at the 10th week sampling.

The average wet weights of oyster meats for each sample are plotted in Fig. 3A, B. With the exception of the 2nd week sample, analysis of variance showed that the mean wet weights of unfed oyster meats were significantly lower (99% confidence) than those of the artificial diet-fed oysters at all sampling periods (Fig. 3B). At the 6th week sampling, the mean wet weights of meats of oysters from diets 2 and 4 were significantly higher (95% confidence) than those from diets 1 and 3. The mean wet meat weight of diet-4 oysters was significantly higher than any other artificial diet at the 12th week sampling, and diet 3 was higher than diet 1.

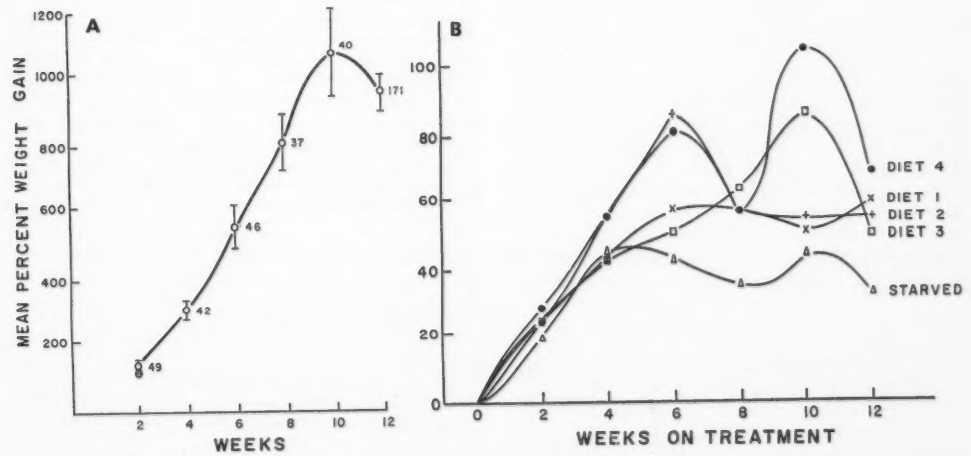

FIG. 2. Mean percent weight gain of individuals sampled from a) natural fed floating tray oysters. Vertical bars indicate two standard errors. The numbers of individuals used to calculate mean percent gain are shown at the right of each point; b) artificial diet fed oysters. Each point is the mean of 20 oysters.

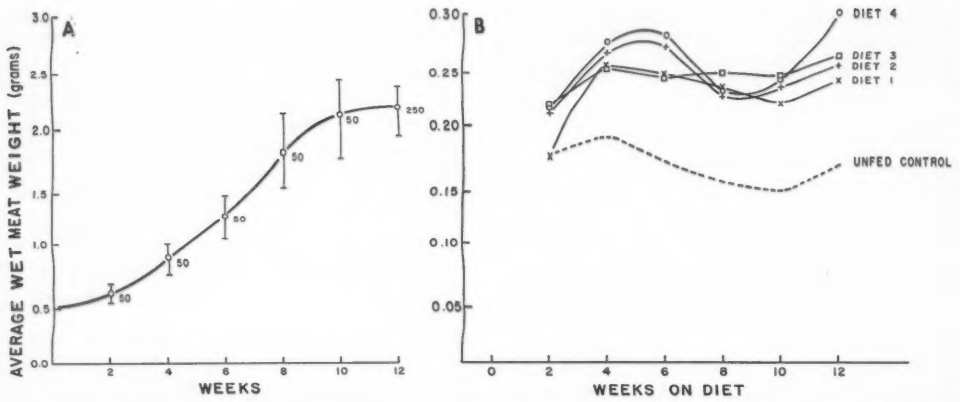

FIG. 3. Average wet weight of meats of oysters sampled from a) natural fed control group. Vertical bars represent two standard errors. Number of individuals sampled is shown to the right of each point; b) artificial diet fed groups. Each point is the average of 20 oysters.

Table 3 gives the mean percent dry weight of the meats and the mean percent edible meat in the whole oysters for the termination samples. In both these sets of data, the natural feed oysters were higher and the unfed controls were lower than the artificial diet fed oysters (95% confidence). The differences between artificial dietary treatments were not as great. The combined average percent edible meat of the cod liver oil (diets 3 and 4) oysters was higher than those receiving corn oil in their diets (1 and 2) (90% confidence).

The mean glycogen content for oysters on each treatment are given in Table 4. The 60% corn starch diets (1 and 3) resulted in higher glycogen values than did the 20% corn starch diets (2 and 4), while cod liver oil (diets 3 and 4) tended generally to produce oysters of higher glycogen content than did the corn oil diets (1 and 2).

TABLE 3. Mean percent of live weight as meat and mean percent dry weight of the meat ±2 SE (12th week samples).

Treatment	Percent dry wt of meat	Percent edible meat
Floating tray	14.96±0.93	16.13±0.63
Diet 1	13.89±0.79	11.71±0.55
Diet 2	12.23±0.36	12.97±0.63
Diet 3	12.26±1.28	13.24±0.72
Diet 4	12.48±0.69	13.09±0.46
Unfed	11.45±0.66	9.85±0.47

Discussion — Previous oyster nutrition studies have emphasized carbohydrates. Haven (1965) reported significant increases in tissue weights when oysters were held in seawater supplemented with wheat or corn starch, but found no detectable influence on mean live-weight or the size of the shell cavity. Gillespie et al. (1966) and Ingole (1967) found that finely ground corn meal produced positive shell growth, increased glycogen deposition, and gains in dry weight. Dunatham et al. (1969) fed various mixtures of ground corn meal, brown rice, barley, crab meat, and other foods and concluded that foods containing between 67.0 and 75.6% car-

TABLE 4. Mean glycogen content of oyster meats ± 2SE (mg/g).

Treatment	Weeks on Treatment				
	2	4	6	8	10
Floating tray	12.2±3.1	14.6±3.4	28.1±4.8	42.2±5.6	42.1± 9.6
Diet 1	7.5±7.9	11.9±4.0	12.0±5.2	16.2±7.5	16.1±12.3
Diet 2	3.4±1.7	8.0±4.0	5.4±1.2	14.6±3.8	17.2±13.3
Diet 3	11.5±2.2	18.4±8.8	16.9±6.6	23.3±3.8	26.0±21.0
Diet 4	10.4±2.8	12.2±5.1	6.7±3.9	14.5±2.1	4.8± 6.4
Unfed	3.5±2.3	3.5±1.6	3.2±0.5	9.3±4.2	4.9± 5.2

bohydrate gave optimal results based on the production of glycogen in the oyster. In all these studies, either single pure carbohydrates such as glucose, or complex carbohydrate sources such as cereal meals, were used as the test diets. In the present study the effect of high dietary carbohydrate levels was confirmed where 60% carbohydrate in the diet was shown definitely to result in greater glycogen production than did the diet containing 20% carbohydrate.

The importance of being able to vary the different components and thus test the effect of single nutrients is illustrated by the work of Dunatham et al. (1969). They found that, although both rice and corn meal contained about 75% carbohydrate, rice consistently produced higher glycogen. Compositional analyses showed marked differences between the amino acid (Block and Weiss 1956), fatty acid (Hilditch and Williams 1964), vitamin and mineral contents of the two cereals. The higher levels of glycogen production when rice meal was fed to oysters might have resulted from one or more of these differences in composition over and above the carbohydrate content.

Differences in the dietary lipid content, provided by the cereals for example, could be one feature giving rise to much of the observed difference. The present study has shown that cod liver oil results in greater glycogen production than does corn oil; it is reasonable to predict that the fatty acid pattern of rice lipid is at least one factor resulting in its superior food value. One characteristic of cod liver is its high content of fatty acids of the linolenic series (recently shown to be essential in the diet of rainbow trout, Castell et al. 1972). Corn oil is characteristically low in linolenic acid and corn meal lipid contains less than half as much linolenic acid as does rice meal lipid (Hilditch and Williams 1964).

The benefit of using more than one criteria in analyzing the nutritional value of any food is again illustrated by the work of Dunatham et al. (1969), who report that although addition of crab meat did not result in significant increases in glycogen production, it did enhance shell growth. In the present study, increasing the level of corn starch in the diet resulted in greater glycogen production, however, when lower levels of starch and higher levels of lipid were fed, the oysters demonstrated more rapid initial weight gains, followed by the observed enlargement of the gonads. This suggests that although diets containing 60–75% carbohydrate result in greater glycogen production, glycogen production by itself is not necessarily the best criterion for determining nutritional value.

It is clear that much more work is necessary to obtain an accurate picture of the oyster's nutritional requirements. The 10-fold faster growth exhibited by the natural diet oysters, compared to the oysters fed artificial diets, demonstrates that the quality and/or quantity of nutrients in the artificial diets is far from optimal. The results for the natural-diet oysters serve as a goal for our continuing studies.

This study has shown that oysters can survive on these artificial diets, and provides a useful basis for future studies of the nutritional requirements of oysters.

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Effects of M.S. 222 Anesthetization on Temperature Selection in Lake Trout, *Salvelinus namaycush*

C. I. GODDARD, J. W. LILLEY, AND J. S. TAIT

Department of Biology
York University, Toronto, Ont.

GODDARD, C. I., J. W. LILLEY, AND J. S. TAIT. 1974. Effects of M.S. 222 anesthetization on temperature selection in lake trout, *Salvelinus namaycush*. J. Fish. Res. Board Can. 31: 100–103.

Yearling lake trout (*Salvelinus namaycush*) were anesthetized with a 150 ppm solution of M.S. 222 for 2 min at 10 C. When tested in a vertical temperature gradient, their behavior was abnormal for 5 days following anesthetization. Initially, they remained at the bottom of the gradient tank, as much as 63% of the time, and when they did swim up into the gradient, their temperature selection was much less precise than that of control fish. The percentage of fish on the bottom declined daily, and on the 6th day their temperature distribution did not differ from that of controls.

GODDARD, C. I., J. W. LILLEY, AND J. S. TAIT. 1974. Effects of M.S. 222 anesthetization on temperature selection in lake trout, *Salvelinus namaycush*. J. Fish. Res. Board Can. 31: 100–103.

Des touladis (*Salvelinus namaycush*) âgés d'un an ont été anesthésiés à l'aide d'une solution de 150 ppm de M.S. 222 pendant 2 minutes à 10 C. Le comportement des poissons placés dans un gradient vertical de températures est anormal pendant 5 jours après l'anesthésie. Au début, ils passent sur le fond du bassin jusqu'à 63% du temps et, quand ils remontent vers le gradient, leur choix de températures est beaucoup moins précis que celui des témoins. Le pourcentage d'observations de poissons sur le fond diminue chaque jour, et, au 6^{e} jour, leur répartition dans le gradient ne diffère pas de celle des témoins.

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DURING research on some aspects of temperature selection we observed that lake trout (*Salvelinus namaycush*) anesthetized with M.S. 222 Sandoz (ethyl *m*-aminobenzoate methanesulfonate) to facilitate identification and weighing were imprecise in their temperature selection for about a week after anesthetization. This observation prompted us to carry out the experiment reported here to measure the extent and duration of the aftereffects of M.S. 222 on temperature selection in lake trout.

Although M.S. 222 has been widely used as an anesthetic in fisheries work in both field and laboratory for almost 2 decades, very little has been reported about its behavioral aftereffects. Its excellent anesthetic properties and convenience at first easily outweighed the detractions of high cost and the possibility, that arises in the use of any anesthetic, of aftereffects on experimental subjects. If aftereffects occurred, they were not readily obvious to early users. Meister and Ritzi (1958) reported that brook trout (*Salvelinus fontinalis*) after an 11-min exposure to a 1000 ppm solution of M.S. 222 "recovered fully in 10 minutes." However, with continued use of M.S. 222, evidence has accumulated that it has at least short-term effects on cardioventilatory activity (McFarland 1959; Randall 1962), blood and tissue chemistry (Wedemeyer 1970; Houston et al. 1971), and carbohydrate metabolism (Crowley and Berinati 1972) of fish. The aftereffect observed in this study is not a change in physiological state of an organ or system but a behavioral effect, a change in the response of the whole animal to an environmental factor.

Materials and methods — Yearling lake trout were obtained from the Laboratory for Experimental Limnology, Maple, Ontario on July 2, 1971, and maintained in a laboratory at York University at 10 C on a day–night light cycle normal for the time of year.

On September 27, 1971, eight lake trout, having a mean weight of 3.9 g, were placed in a 10 C, 150 ppm solution of M.S. 222 for 2 min. In this time, five fish reached stage IV of anesthetization (McFarland 1959), showing complete loss of equilibrium and muscle tone and cessation of opercular movements. Three reached stage III, showing loss of equilibrium and muscle tone but retaining slow, shallow opercular movements. The eight fish were then removed from the solution and placed in a temperature gradient tank.

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The preferred temperatures of the treated and the control fish were determined in a 405-liter vertical gradient tank similar to that described by Brett (1952) with the addition of an aluminum cooling coil, as described by Garside and Tait (1958), in the bottom zone.

To set up the temperature gradient, the tank was filled with 4–5 C water, and then hot water was directed down through the rectangular aluminum coil suspended in the tank. When the top zone had warmed to 14–15 C, the fish were transferred from the acclimation tank to the gradient tank. During the next 1–1.5 hr, the gradient was further established, and the fish adjusted to the tank and began swimming about. Diffuse lighting was provided by four 40-w fluorescent lights in two ceiling fixtures located 3 and 5 m away from the tank. Incident light on the center of the glass front of the tank was 35 lx.

Observations on the distribution of the fish in the 10 depth zones of the tank were made every 15 sec for 5 min. Such a set of observations was termed a "run." The gradient was usually shifted slightly during the 30-min interval between runs. Temperature readings from thermometers located in each zone were taken before and after each run and averaged to give the mean temperature of each zone. Observations on 15 control fish were made on September 15 and October 5, 1971 and on the treated fish on the day on which they were anesthetized, then daily for 6 days and finally on October 6, 9 days after treatment.

Results — The 10 runs done each day were combined to give a frequency distribution in class intervals of 1 degree C and each day's results were plotted as a percentage frequency histogram (Fig. 1).

When placed in the vertical gradient tank, the control lake trout reacted in the way described by McCauley and Tait (1970). They rested on or near the bottom for a few minutes, swam to the surface where they filled their swimbladders, and then swam leisurely about the tank, concentrating in a few zones. Their preferred temperature was 11.5 C, determined as the mode, or 11.6 $\pm$ 0.02 C as the arithmetic mean of the combined data.

The reaction of the eight treated lake trout was very different from that of the controls. Figure 1 shows that the distribution of the fish in the tank was affected in two ways. Firstly, there was a tendency for fish to be inactive and to remain on the bottom of the tank, and secondly, those fish that moved off the bottom and began swimming hrough the temperature gradient either failed to select a temperature or lacked precision in their selection. Six days elapsed before these two after-effects of anesthesia disappeared.

When first placed in the gradient tank, the fish were still in stage III or IV of anesthesia and they settled slowly to the bottom. Within a few minutes they had resumed normal opercular movements and within 1 hr all had recovered their equilibrium and muscle tone, but they were still resting on the bottom or maintaining their position just above it. While the observations that constitute a run normally are begun 1–1.5 hr after the fish are introduced to the gradient, the first run was delayed 3.5 hr, and even then five of the fish were still on or near the bottom. The other three were swimming about slowly in the tank, having filled their swimbladders at the surface. In each of the 10 runs more than half of the fish were on the bottom. When the runs were

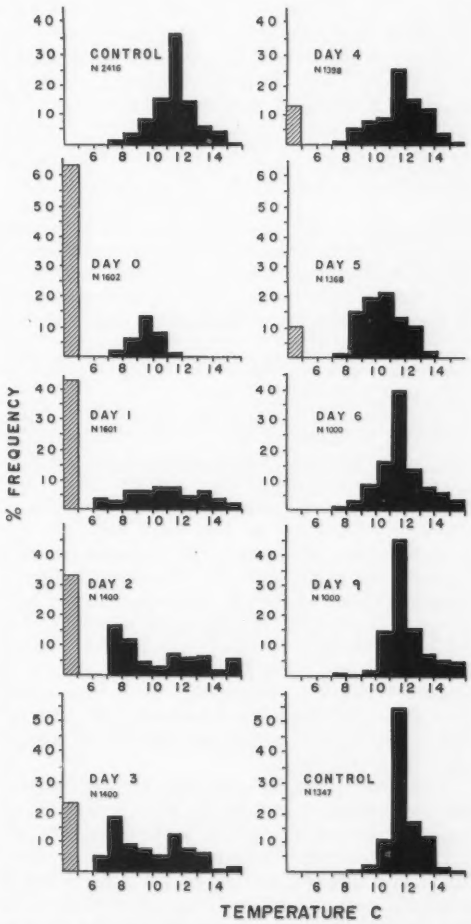

FIG. 1. Percentage frequency distributions of lake trout (*Salvelinus namaycush*) treated with M.S. 222 and controls in vertical temperature gradients. Each histogram represents the combined data from observations made in 1 day. Cross-hatched bars represent observations of fish at the bottom of the tank. N = number of observations.

combined (Fig. 1, day 0), 63.5% of the observations were in the bottom zone. The rest of the fish appeared to be distributed around a mode of 9.5 C. On day 1 (24 hr after anesthetization), the first run was started 1.5 hr after introduction of the fish. Five fish had swum up and were swimming normally about the tank. The other three fish remained on the bottom throughout the 10 runs. Percentage frequency of fish in the bottom zone was 43.3, and in succeeding days it continued a steady decline to zero by day 6. On days 1–3, distribution of those fish that were swimming in the gradient was apparently random, with virtually no indication of temperature selection. Normal distributions began to reappear on day 4, and by day 6 the distribution was the same as that of the controls with a sharply defined mode at 11.5 C and a mean preferred temperature of 11.6 ± 0.05.

Discussion — The performance of the control lake trout in this experiment reaffirms the repeatability of preferred temperature determinations for this species. The mean preferred temperature of 11.6 C does not differ significantly from the final preferendum of 11.7 C or from the value of 11.8 for lake trout acclimated to 10 C recorded by McCauley and Tait (1970).

However, observations reported here show clearly that M.S. 222 caused lake trout to deviate markedly from their preferred temperature range and to behave abnormally in a temperature gradient tank in at least two ways. The first observed abnormality, a tendency to stay on the bottom of the tank, was a prolongation of a normal or expected behavior pattern. Since the fish were transferred directly from anesthetic solution to the tank, recovery took place on the bottom. From past experience in using M.S. 222 on fish, we have observed that fish are sluggish or less active for a few hours after their apparent recovery. However, we did not realize that the sluggishness lasted beyond a day and did not expect fish to remain on the bottom several days after treatment.

Lake trout normally show little tendency to stay on the bottom, therefore the high proportion of treated fish remaining on the bottom suggests that there are lingering effects of the anesthetic which result in a general lassitude and a suppression of normal swimming activity. There is no evidence from this observed behavior of an effect on temperature sense. The effect on preferred temperature is a removal of some test individuals from participation.

The second observed abnormality, total or partial failure of the fish to produce a normal curve distribution with respect to temperature, indicates that M.S. 222 interferes either with temperature reception or with the nervous coordination of movements that bring the fish into or keep them in some temperature zones more than others. Either sensory receptors or central nervous system mediators or both are involved.

The loss of three fish, one after day 1 and two after day 5, resulting in smaller N values (Fig. 1), raises the question whether the apparent return of treated fish to normal behavior was partly caused by the loss of these individuals. The fish were not found dead and it is assumed that they escaped from the holding tank. Inspection of the hatched bars in Fig. 1 shows a smooth decline in frequency of fish on the bottom of the tank. There is no evidence that loss of fish affected frequency except perhaps in hastening the drop from 10 to 0% between days 5 and 6. Precision of selection did not improve between days 1 and 2 and it had improved substantially before the loss of two fish after day 5. We conclude that the loss of fish did not significantly affect the results of the experiment.

A small number of papers have reported effects of chemical agents on preferred temperature of fish. Ogilvie and Fryer (1971) treated guppies (*Poecilia reticulata*) with sodium pentobarbital at a dosage lower than that required to produce anesthesia, and observed their temperature selection for 4 hr. The response of guppies was similar to that of the lake trout in the present experiment in that each appeared to have a breakdown of the process by which they select temperature. Initially, the distribution of guppies was erratic and the mean was depressed by 6 degrees C. After 4 hr, selection was approaching normal. The great difference in the duration of effect in guppies and lake trout may be due to the difference in dosage and type of anesthetic used. Ogilvie and Anderson (1965) noted considerable imprecision in the temperature distribution of Atlantic salmon treated with DDT. They attributed this imprecision to variability between experiments in the amount of DDT freely suspended in the water rather than to failure of the fish to select precisely. Whatever the interpretation, the effect is far less pronounced than that observed in guppies and lake trout under the conditions discussed above. The major effect on the salmon was an upward shift of up to 10 degrees C in the mean selected temperature. The treated lake trout distributions showed so little central tendency until day 4 that calculated means have little meaning. However, the fish tended to be in lower temperature zones, and means, calculated but not shown, moved erratically up from 9.5 C on day 0 to become stable at the control level by day 6.

The observed behavioral aftereffects on lake trout had about the same duration as the long-term effects

of M.S. 222 on brook trout reported by Houston et al. (1971). They found that plasma glucose levels and tissue levels of potassium were raised and tissue levels of calcium were lowered for up to 8 days after exposure to surgical procedures and a 100 ppm solution of M.S. 222 for 15 min. (According to Crowley and Berinati (1972), a 2-min exposure does not affect glucose levels.) Houston et al. favor the view that the more persistent changes were related to general endocrine responses to handling procedures rather than to M.S. 222 which apparently is cleared from body tissues in a matter of hours. In our experiment, the abnormal behavior of the lake trout is attributable to M.S. 22, the only variable. This implies that M.S. 222 either persists in effective traces beyond the few hours that it can be detected chemically or initiates a sequence of responses that lasts for several days.

We had not used M.S. 222 previously on fish that were to be tested for temperature selection and any caution exercised in the use of M.S. 222 may have been justified. Anyone using M.S. 222 on fish prior to behavioral studies should allow at least a week to avoid aftereffects of the anesthetic.

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First Record from North America of the Piscicolid Leech, *Ozobranchus margoi*, a Parasite of Marine Turtles

RONALD W. DAVIES

Department of Biology
University of Calgary, Calgary T2N 1N4, Alta.

AND C. G. CHAPMAN

Wometco Miami Seaquarium, Miami, Fla. USA

DAVIES, R. W., AND C. G. CHAPMAN. 1974. First record from North America of the piscicolid leech, *Ozobranchus margoi*, a parasite of marine turtles. J. Fish. Res. Board Can. 31: 104–106.

Ozobranchus margoi is recorded from North America for the first time, as an ectoparasite of the marine turtles *Chelonia mydas mydas*, *Caretta caretta caretta*, *Eretomochelys imbricata imbricata*, and *Lepidochelys kempii*. The pathology and treatment of the leech infestation are described and the presumed distribution of the species compared with that of *Ozobranchus branchiatus*, the only species of the genus previously recorded from North America.

DAVIES, R. W., AND C. G. CHAPMAN. 1974. First record from North America of the piscicolid leech, *Ozobranchus margoi*, a parasite of marine turtles. J. Fish. Res. Board Can. 31: 104–106.

Les auteurs signalent pour la première fois en Amérique du Nord *Ozobranchus margoi*, ectoparasite des tortues marines *Chelonia mydas mydas*, *Caretta caretta caretta*, *Eretomochelys imbricata imbricata* et *Lepidochelys kempii*. Ils décrivent les manifestations pathologiques et le traitement de l'infestation par cette sangsue, et comparent ce qu'ils croient être la répartition de l'espèce avec celle d'*Ozobranchus branchiatus*, la seule espèce du genre déjà signalée en Amérique du Nord.

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IN April 1973 an infestation of leeches identified as *Ozobranchus margoi* was discovered on the marine turtles in a pool at the Wometco Miami Seaquarium (Florida, USA); the infestation was first noted when the turtles continually attempted to haul out. Of the 25 specimens of *O. margoi* examined in detail, all appeared to be sexually mature, with the united genital pores visible. They ranged in length from 18 to 25 mm in comparison with published records of 15–30 mm (Apathy 1890) and 17–26 mm (Cordero 1929). *Ozobranchus branchiatus* is much smaller, ranging in length from 5 to 11 mm (Ghosh et al. 1963).

The pool containing the infected turtles was circular (15 m in diameter), encircling an island, and filled with filtered bay water pumped in at a high turnover rate. There appeared to be no preference by *O. margoi* as to host species, all 85 turtles in the pool being infected. The host species were mostly Atlantic green turtles (*Chelonia mydas mydas*) and Atlantic loggerhead turtles (*Caretta caretta caretta*), the others being several Atlantic hawksbill turtles (*Eretomochelys imbricata imbricata*) and Atlantic Ridley turtles (*Lepidochelys kempii*). The largest loggerhead turtles were the most heavily infected. However, *O. margoi* was not found on various species of local terrestrial turtles on the island or on various numbers of small lemon sharks, nurse sharks, tarpons, southern stingrays, assorted Atlantic reef fishes (parrotfish, black durgons, trunkfish), and spiny lobsters. An adjacent pool also containing the same species of turtles and on the same water supply, except that the direction of water flow was towards the affected pool, has not harbored *O. margoi*.

On all species of turtles, *Ozobranchus margoi* was always found on the ventral surface of the flippers, the neck, the external cloaca, and the corners of the mouth, and in the folds of the soft tissue between the carapace and the plastron (Fig. 1). No leeches were found on the carapace. The numbers found on individual turtles varied greatly but in many cases 800–900 were estimated. Large clumps of eggs covered by a chitinous membrane were also commonly observed attached to the plastron (Fig. 2).

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FIG. 1. The distribution of *Ozobranchus margoi* on the posterior flippers, on the external cloaca and in the folds of soft tissue between the carapace and plastron on *Caretta caretta*.

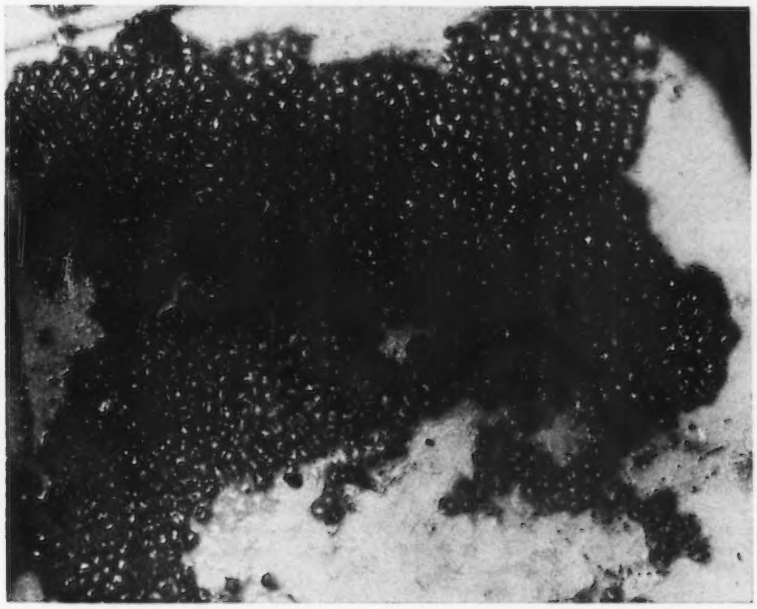

FIG. 2. Egg clusters of *Ozobranchus margoi* on the plastron of *Caretta caretta*.

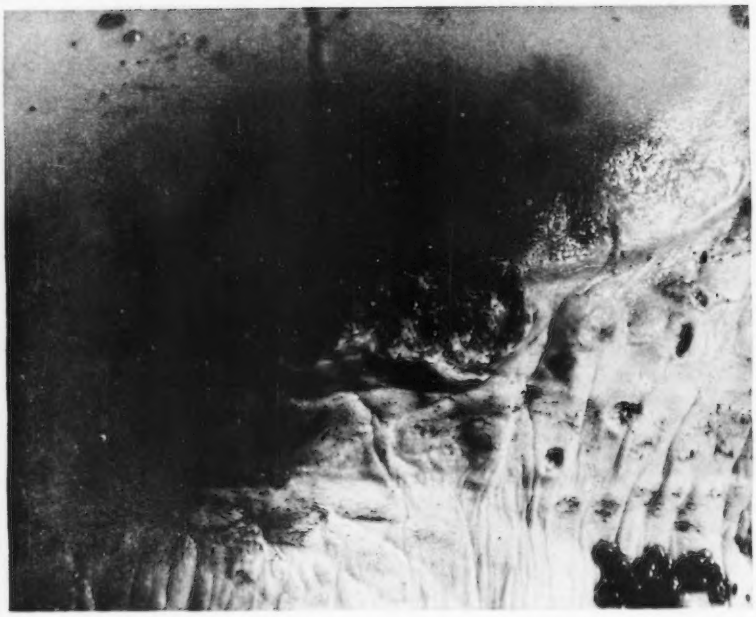

FIG. 3. Lesions at the sites of attachment of *Ozobranchus margoi* on the plastron of *caretta*.

Davies — J. Fish. Res. Board Can.

The only pathology observed was the excavation and destruction of the plastron at the sites of attachment. (Fig. 3). There were no signs of the fibro-epithelial tumors observed by Nigrelli and Smith (1943) and Hendrickson (1958) at the sites of attachment of *O. branchiatus* on *Chelonia mydas*. All the specimens of *O. margoi* examined had blood in their guts; samples of this blood and blood taken directly from turtles were examined unsuccessfully for the presence of blood parasites.

Treatment was attempted in two ways. Firstly, 10% formalin was applied directly to the leeches and egg masses of beached turtles, which were subsequently kept out of the water for at least 3 hr. This treatment was not highly effective. Secondly, copper sulphate was added to the pool through the inlet pipe and a 0.3 ppm concentration maintained in the pool (monitored with a spectrophotometric method) for 6 hr. This procedure was repeated twice, 7 days apart, and both the leeches and their egg clumps were eliminated from the turtles. Three months after treatment, *O. margoi* was again found in low numbers on most turtles.

Of the 86 infected turtles three died: one large loggerhead, although no internal lesions were discovered; and two Ridleys, the day after the completion of the copper sulphate treatment, whether as a result of the infestation or of the treatment being unknown.

The genus *Ozobranchus* de Quatrefages, 1852, is represented by six species, all of which are primarily ectoparasites of chelonids. The only species previously recorded from North America, *Ozobranchus branchiatus* (Menzies 1791), was collected from *Chelonia mydas* (Linn.) from Key West Florida (MacCallum and MacCallum 1918) and from the west coast of Florida (Nigrelli and Smith 1943). *Ozobranchisus branchiatus* has also been recorded from the Pacific Ocean between the tropics (Menzies 1791), Australia (Baird 1869), Japan (Oka 1910), Sumatra (Hendrickson 1958), India (Ghosh et al. 1963), and the Malay Peninsula and Sarawak (de Silva and Fernando 1965).

Ozobranchus margoi (Apathy 1890) was originally described from specimens collected from *Thalassochelys corticata* caught in the Bay of Naples (Mediterranean), the location from which Selensky (1915) described the species. Oka (1927) recorded it as an ectoparasite of both a dolphin (*Delphinus longirostris*) and the marine turtle *Caretta olivacea* (= *Lepidochelys olivacea* Escholtz) off the coast of Kyushu (Japan), Cordero (1929) recorded *O. margoi* as an ectoparasite of *Thallassochelys caretta* (= *Caretta caretta caretta* Linn.) off the coast of Montevideo (Uruguay), and Richardson (1969) recorded it on *Chelonia mydas* from Heron Island, Australia.

Though *O. margoi* and *O. branchiatus* are now clearly distinguished on the basis of five and seven pairs, respectively, of digitiform branching branchiae, some confusion has occurred in the past. Menzies (1791), in describing *Hirudo branchiata* (=*Ozobranchus branchiatus*) as having "a row of soft pellucid branchy bristles on each side, opposite to one another, making in all seven pairs," showed only five pairs of branchiae in his illustration of the species. Oka, in 1910, described *O. branchiatus* in Japan as having either five or seven pairs of branchiae, and in 1912 he recorded only *O. branchiatus* in Japan while clearly distinguishing between *O. branchiatus*, *O. margoi*, and *O. jantseanus* Oka 1912; yet, in 1927, he recorded *O. margoi* for the first and only time from Japan. The paucity of distributional data combined with this possible confusion between *O. branchiatus* and *O. margoi* makes speculation on the distributional patterns for both species tenuous. Since many of the green turtles in the Wometco Seaquarium were captured on Ascension Island in 1970 while all the other species were caught locally, it is not possible to know whether *O. margoi* occurs naturally in Florida waters or is a recent ntroduction. Evidence from the distribution of *O. branchiatus* and the more limited distributional evidence for *O. margoi* would indicate that *O. margoi* might be expected in all warm water areas in the distribution of the host species.

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Distribution of LDH-B″ Gene in Resident and Anadromous Rainbow Trout (*Salmo gairdneri*) from Streams in British Columbia

L. HUZYK AND H. TSUYUKI

Department of the Environment, Fisheries and Marine Service
Vancouver Laboratory, Vancouver, B.C. V6T 1X2

HUZYK, L., AND H. TSUYUKI. 1974. Distribution of LDH-B″ gene in resident and anadromous rainbow trout (*Salmo gairdneri*) from streams in British Columbia. J. Fish. Res. Board Can. 31: 106–108.

The B″ subunit of lactate dehydrogenase from the liver tissue of wild populations of resident rainbow trout (*Salmo gairdneri*) from the Loon and Pennask Lake areas has been found in a frequency of 0.400–0.800. An electrophoretically identical subunit was found in similar proportions in anadromous steelhead trout from the Thompson River, and from the Coquihala River in considerably smaller proportions. Steelhead from some coastal streams in and near Vancouver and Vancouver Island, and other selected streams from the Fraser and Skeena river systems generally revealed a very low frequency of less than 0.1.

HUZYK, L., AND H. TSUYUKI. 1974. Distribution of LDH-B″ gene in resident and anadromous rainbow trout (*Salmo gairdneri*) from streams in British Columbia. J. Fish. Res. Board Can. 31: 106–108.

La sous-unité B″ de la lactase déshydrogénase du tissu hépatique de populations sauvages de truites arc-en-ciel (*Salmo gairdneri*) résidant dans la région des lacs Loon et Pennask se trouve dans la fréquence de 0.4800–0.800. Une sous-unité électrophorétiquement semblable a été trouvée dans les mêmes proportions chez la truite steelhead de la rivière Thompson, et dans des proportions beaucoup plus faibles dans la rivière Coquihala. Les truites steelhead de certains cours d'eau côtiers de Vancouver et de l'île Vancouver et environs, ainsi que d'autres cours d'eau choisis du régime du fleuve Fraser et de la rivière Skeena montrent généralement une très basse fréquence, inférieure à 0.1.

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ALLELIC LDH subunit, now designated B″ (Baily and Tsuyuki unpublished data; Tsuyuki and Williscroft 1973), and first found in the liver of rainbow trout (*Salmo gairdneri*) was described by Williscroft and Tsuyuki (1970). Phenotypes possessing this subunit were found in high frequency in resident rainbow trout from Kokanee Creek (Northcote et al. 1970). Kinetic differences between LDH-B_4' from the liver of the uncommon B″ phenotypes and LDH-B_4' from that of the common B′ phenotypes have been described (Tsuyuki and Williscroft 1973). While these properties in themselves are interesting, the observation that the B″ phenotypes predominate among trout that live in waters characterized by faster flow (Northcote et al. 1970) or that LDH-B_4' has a considerably higher rate of conversion of lactate to pyruvate in vitro is even more significant. The above may mean that the

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LDH-B_4'' phenotype possesses superior swimming qualities, and indeed, subsequent studies are supporting this view. Therefore we undertook and now report a survey of the incidence of LDH-B_4'' in anadromous and nonanadromous fish populations living in selected British Columbia streams. The potential role that the B″ rainbow trout phenotypes could play in future propagation of this species as well as its anadromous variety is one of the prime reasons for this study.

Materials and methods — Mature rainbow trout from the inlet and outlet streams of Pennask and Loon Lakes, Hihium Creek and Thunder Creek, were collected between September 1970 and July 1971 by seining or by dip-netting. The liver tissues of steelhead trout from the Thompson, Vedder, Cheakamus, Squamish, Dean, Coquihala, Seymour, Capilano, Mamquam, Coquitlam, Silver, Nahatlach, Oyster, Salmon, Nanaimo, Gold, Bulkley, Johanson, Copper, Sustut, Upper Sustut, Morice, and Clean Bright rivers, were collected from the angling catch of a number of steelheaders during 1970–73.

Lactate dehydrogenase was prepared from the frozen liver tissues by homogenizing with 5 vol of chilled 0.05 M phosphate buffer, *p*H 7.55, and by centrifuging at 27,000 × g. The LDH phenotypes were identified by micro starch gel electrophoresis as described previously (Williscroft and Tsuyuki 1970).

Results and discussion — The starch gel electrophoretic mobility patterns of the liver LDH from rainbow trout are not shown here as the zymograms are similar to those published earlier (Williscroft and Tsuyuki 1970; Northcote et al. 1970) for the same species. Steelhead trout zymograms are also not shown as the liver LDH mobilities are identical to those of rainbow trout (Tsuyuki and Williscroft 1973).

The distribution of LDH phenotypes for rainbow and steelhead trout are shown in Table 1. Except for Thunder Creek, chi-square tests for the individual areas indicate no significant deviations between the observed and expected values calculated from the Hardy-Weinberg distribution. Wild populations of rainbow trout from the inlet and outlet streams to Loon Lake, and from Hihium and Thunder Creeks all analyzed to qB″ ranging from 0.395 to 0.552. There is no obvious difference between

TABLE 1. Distribution of LDH phenotypes in rainbow trout and steelhead trout (*Salmo gairdneri*) from British Columbia.

Area	Total no.	Phenotypes B″B″ obs.	B″B″ exp.	B″B′ obs.	B″B′ exp.	B′B′ obs.	B′B′ exp.	Frequencies qB″	Frequencies qB′	X^2	Probability of worse fit (2 df)
Rainbow trout											
Loon Inlet	172	34	37.2	92	85.6	46	49.2	.465	.535	.962	.750–.500
Loon Outlet	29	10	8.9	12	14.3	7	5.8	.552	.448	.739	.750–.500
Hihium Creek	13	1	2.3	9	6.4	3	4.3	.423	.577	2.184	.500–.250
Below Hihium Creek	19	3	3.0	9	9.1	7	6.9	.395	.605	.002	1.00 –.995
Thunder Creek	23	1	4.8	19	11.4	3	6.8	.457	.543	10.1	.010–.005
Pennask Outlet	18	6	6.1	9	8.8	3	3.1	.583	.417	.010	.995–.990
Pennask Inlet	20	12	12.8	8	6.4	0	0.8	.800	.200	1.250	.750–.500
Steelhead trout											
Thompson River	107	27	23.3	46	53.3	34	30.4	.467	.533	2.014	.500–.250
Vedder River	56	0	0.4	9	8.2	47	47.4	.080	.920	.481	.900–.750
Cheakamus River	37	0	0.2	5	4.7	32	32.1	.068	.932	.219	.900–.750
Squamish River	56	0	0.1	4	3.9	52	52.0	.036	.964	.103	.950–.900
Dean River	26	0	0	0	0	26	26	0	1.00	0	1.00
Coquihala River	10	1	0.4	2	3.2	7	6.4	.20	.80	1.406	.500–.250
Seymour River	14	0	0	0	0	14	14	0	1.00	0	1.00

[a]Additional steelhead trout caught in British Columbia streams: Capilano R. (eight samples), Mamquam R. (three), Coquitlam R. (three), Silver R. (eight), Nahatlach R. (two), Oyster R. (one), Salmon R. (one), Nanaimo R. (one), Gold R. (one), Bulkley R. (three), Johanson Cr. (one), Copper R. (one), Sustut R. (five), Upper Sustut (one), Morice R. (two), Clean Bright R. (one).

[b] A mutant, very slightly anodal to B′ under our electrophoretic conditions, was observed in 10 liver samples (nine from the Cheakamus-Squamish area, one from the Seymour River), and has been included in B′ frequency calculations.

samples from streams flowing into and out of Loon Lake. Samples from Pennask Lake area generally revealed a higher qB″, particularly the inlet specimens, than those from Loon Lake. So far as we know, Kokanee Creek (Williscroft and Tsuyuki 1970; Northcote et al. 1970) in the watersheds of the Columbia River and Loon and Pennask Lake regions in the Thompson River watershed are the only areas of abundance of the B″ phenotype of rainbow trout. Although further surveys for the B″ gene in rainbow trout were not carried out, the scarcity of the B″ phenotype in steelhead from a number of British Columbia streams suggests that this gene is not too common. Other electrophoretic studies (Massaro and Markert 1968; Bouck and Ball 1968) have not revealed further sources.

The frequency of the occurrence of the B″ gene in steelhead (Table 1) is divided sharply into two groups. Steelhead from the Thompson River represent the only ones with qB″ frequency of 0.47, a value which is in the range of those found for rainbow trout in upper watersheds of the river. The Vedder, Cheakamus, Squamish, and Dean rivers, where significant numbers of steelhead liver samples were available, all disclosed a qB″ frequency of less than 0.10. Liver samples from the Seymour and Capilano rivers, numbering 14 and eight, respectively, showed no B″gene. Two heterozygotes were obtained out of eight winter steelhead from Silver Creek. Of 10 winter steelhead from the Coquihala River (Table 1), one B″B″ homozygote and two B″B′ heterozygotes were obtained. The Coquihala is the only other river besides the Thompson which may yield the B″B″ homozygotes in useful numbers. From a total of 26 steelhead liver samples collected from 14 other streams (Table 1, footnote) no B″B″ homozygote and only four heterozygotes were found. Fourteen of these 26 samples from seven different streams in the Skeena river system yielded only one heterozygote. The number of samples from any one stream, however, is so small that further interpretation is not appropriate.

Since two types, the summer and winter steelhead, are recognized (Smith 1960, 1969; Withler 1966), the possibility that the B″ gene is a summer steelhead characteristic was considered. Although all samples from the Vedder, Cheakamus, and Squamish Rivers are authentic winter steelhead, a significant proportion of the Thompson River steelhead caught during October was similar to that of later samples. By direct evidence the 26 steelhead liver samples from the summer steelhead from the Dean River (Table 1) revealed no B″ gene. At least in one instance, the B″ phenotype is not associated with summer steelhead.

Preliminary evidence from this laboratory indicates a direct correlation of the B″B″ phenotype of rainbow trout with superior swimming endurance. Although it does not follow that because the same phenotype is found in steelhead trout the same correlation applies, the implication warrants further investigation. The source of and the method for selecting the B″B″ phenotypes of both rainbow trout and steelhead trout have been identified should future needs decide on the propagation of these strains.

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Immature Insects (Plecoptera, Trichoptera, and Ephemeroptera) Collected from Deep Water in Western Lake Superior[1]

JAMES H. SELGEBY

U.S. Bureau of Sport Fisheries and Wildlife Ashland, Wis. 54806, USA

SELGEBY, J. H. 1974. Immature insects (Plecoptera, Trichoptera, and Ephemeroptera) collected from deep water in western Lake Superior. J. Fish. Res. Board Can. 31: 109–111.

Five species of aquatic insects — two plecopterans, two trichopterans, and one ephemeropteran — usually found in streams or ponds were collected in water 32–100 m deep in western Lake Superior. All appear to be new records for the lake and all were collected from far greater depths than previously recorded for these forms.

SELGEBY, J. H. 1974. Immature insects (Plecoptera, Trichoptera, and Ephemeroptera) collected from deep water in western Lake Superior. J. Fish. Res. Board Can. 31: 109–111.

Cinq espèces d'insectes aquatiques — deux plécoptères, deux trichoptères et un éphémère — ordinairement trouvés dans des cours d'eau ou des étangs ont été capturés à des profondeurs de 32 à 100 mètres dans le lac Supérieur occidental. Ce semble être des mentions nouvelles pour ce lac, et toutes les espèces ont été capturées à des profondeurs beaucoup plus grandes que celles déjà signalées pour ces formes.

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THIS report presents information on five species of aquatic insects collected during routine sampling in the Apostle Islands region of Lake Superior, in water 32–100 m deep. The limited published information on the aquatic insects of Lake Superior consists mostly of observations in relatively shallow water. The species discussed here — two plecopterans, two trichopterans, and one ephemeropteran — appear to be new records for the lake.

Materials and methods — Most of the insects collected were found clinging to gillnets or entrapped by debris in the cod ends of bottom trawls. Mayfly nymphs were taken in special sampling devices that consisted of hardware-cloth boxes, about 20 × 20 × 40 cm, containing beach stones. Six samplers were lowered on buoy lines to depths of 25–100 m on a steep bank at station 9 (Fig. 1) on June 23, 1972, and recovered on August 9.

Specimens were obtained only from water 32–100 m deep (Table 1) although gillnets and trawls were often fished in shallower water and in other areas of the lake. Most of the specimens were collected from areas with a stony bottom, although two trichopterans collected at

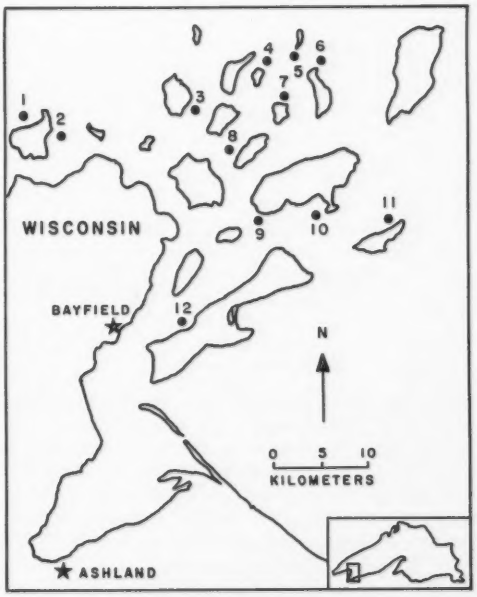

FIG. 1. The Apostle Islands region of western Lake Superior, showing the 12 stations (Table 1) where aquatic insects were collected in 1971–72.

[1]Contribution 477, Great Lakes Fishery Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, Ann Arbor, Mich. 48107, USA.

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100 m came from a trench bottom covered with organic debris. None of the species were taken in extensive sampling with benthic grabs.

Species — The Plecoptera include 64 nymphs of *Isoperla bilineata* (Say) and a single female nymph of *Isogenus* (*Isogenoides*), probably *Isogenos krumholzi* Ricker. *Isoperla bilineata* is common in some large Wisconsin streams, although it has not previously been recorded from the northern part of the state (W. L. Hilsenhoff personal communication). *Isogenus krumholzi* is known from adults taken in Minnesota and Michigan, but its nymph has not been described (W. E. Ricker personal communication). Although the specimen collected could not be positively separated from *Isogenus olivaceus* Walker, it differed in the banding of the anterior abdominal segments (W. E. Ricker personal communication) and in size — it was larger than *I. olivaceus* collected in Wisconsin (W. L. Hilsenhoff personal communication).

The larval Trichoptera included 29 *Phryganea cinerea* Walker and 10 *Agrypnia straminea* Hagen, although several of the specimens of *Agrypnia* may be of a different species whose key characteristics are unknown (G. B. Wiggins personal communication). *Phryganea cinerea* and *A. straminea* are northern species that frequent glacial lakes and marshes (Ross 1944). In Wisconsin, species of *Phryganea* and *Agrypnia* are fairly common in lakes, ponds, and sluggish streams (Hilsenhoff 1970).

The Ephemeroptera nymphs are probably *Heptagenia pulla* (Clemens), although they were too young for positive identification. In Wisconsin, *H. pulla* has been collected in two northern rivers tributary to Lake Superior, and on rocks near the shoreline of Green Bay, Lake Michigan (W. L. Hilsenhoff personal communication).

Characteristics of the habitat — The five species were all collected from depths far greater than those at which they have previously been reported.

TABLE 1. Dates and localities of collection for five species of aquatic insects, Apostle Islands region of Lake Superior, 1971–72.

Order, species, and date	Number collected	Station number (Fig. 1)	Depth (*m*)	Gear[a]
Plecoptera				
Isoperla bilineata				
May 11, 1971	1	10	36–43	G
June 30, 1971	7	1	32–50	G
July 1, 1971	11	3	32–45	G
July 8, 1971	33	4,7	32–45	G
July 9, 1971	9	9	35–68	G
Aug. 11, 1972	3	9	36–48	G
Isogenus (*Isogenoides*) *krumholzi*[b]				
May 11, 1971	1	10	36–43	G
Trichoptera				
Agrypnia straminea				
July 1, 1971	1	3	32–45	G
July 8, 1971	9	5	34–41	G
Phryganea cinerea				
May 11, 1971	2	10	36–43	G
June 28, 1971	7	2	36–43	G
Nov. 8, 1971	1	8	43	T
Nov. 16, 1971	10	6	45	T
Nov. 17, 1971	6	12	36	T
July 17, 1972	1	10	54	T
Aug. 31, 1972	2	10	100	T
Ephemeroptera				
Heptagenia pulla[b]				
Aug. 9, 1972	2	9	50	S

[a]G = gillnet; T = trawl; S = substrate sampler.
[b]Tentative identification.

The following characteristics may contribute to the suitability of the deepwater environment for these insects in the Apostle Islands region: currents among the islands are frequently swift; the water is clear (average Secchi disc reading about 6–10 m); dissolved oxygen is always near saturation; and water temperature fluctuates only from about 4 to 6 C at the depths of collection. The heavily wooded islands and mainland shore provide a substantial quantity of allochthonous organic material which serves as case-building material (for Trichoptera) or as food.

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Interaction of Free Fatty Acids with Fish Muscle in Vitro[1]

E. A. CHILDS[2]

Seafoods Laboratory
Department of Food Science and Technology, Oregon State University, Astoria, Oreg. 97103, USA

CHILDS, E. A. 1974. Interaction of free fatty acids with fish muscle in vitro. J. Fish. Res. Board Can. 31: 111–113.

When fresh fish muscle (*Ophiodon elongatus*) was exposed to free fatty acids (safflower oil hydrolysate), the whole myofibril lost its suspensibility more readily than its component proteins lost their solubility. SDS-acrylamide gel electrophoresis indicated that tropomyosin was the most readily insolubilized protein. In the presence of 0.04% formaldehyde, effects of the free fatty acids were increased approximately threefold.

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Quand on expose le muscle de poisson (*Ophiodon elongatus*) à des acides gras libres, la myofibri le entière est insolubilisée plus facilement que ses protéines constituantes. L'électrophorèse sur gel SDS-acrylamide démontre que la tropomyosine est la protéine myofibrillaire le plus facilement insolubilisée. En présence de formaldéhyde à 0.04%, les effets des acides gras libres sont à peu près triplés.

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IN VITRO experiments have shown that addition of small amounts of C_{18} fatty acids can reduce the quantity of extractable salt-soluble protein from fish muscle (Anderson et al. 1963). It was later suggested (Anderson and Steinberg 1964) that intact lipids could protect fish muscle proteins from insolubilization induced by free fatty acids (FFA). Conversely, Connell (1968) referred to unpublished data which indicated no relationship between salt-soluble protein extractability and either the ratio of FFA to intact lipid or the disposition of lipid in the tissue. Recently, it was suggested (Castell 1971) that in lean fish FFA might interact directly with myofibrillar protein during oxidation. In addition to decreases in extractable salt-soluble proteins, a decrease in the amount of isolable whole

[1]Technical Paper No. 3611, Oregon State University Agricultural Experiment Station.

[2]Present address: Department of Food Technology and Science, University of Tennessee, Knoxville, Tenn. 37901, USA.

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myofibril units during frozen storage has recently been demonstrated (Childs 1973b).

Since the myofibrillar portion of the salt-soluble protein fraction does not exist as individual proteins, but rather as part of the whole myofibril complex, the interaction of FFA with the whole myofibril was investigated in lingcod (*Ophiodon elongatus*). The objectives were to determine the effects of in vitro exposure to free fatty acids in a safflower oil hydro lysate on amounts and protein complements of extractable salt-soluble proteins and isolable whole myofibrils, and also the effects of simultaneous exposure to FFA and formaldehyde (FM) on saltsoluble proteins and whole myofibrils.

Experimental — Lingcod were obtained 1 day after capture (Feb. 1973), and the white axial muscle dissected and held on ice until experimentation on the same day.

Prior to extraction of salt-soluble proteins, all connective tissue in the portion of muscle to be analyzed was dissected and discarded. The salt-soluble proteins were then extracted and whole myofibrils were isolated by procedures described previously (Childs 1973a, b). During isolation of whole myofibrils, a 0.025 M KCl, 0.039 M boric acid, 0.005 M disodium EDTA adjusted to *p*H 7.1 buffer was used for all steps through the first clarification (400 *g* for 3 min).

The safflower oil hydrolysate was added to the initial extraction buffer-minced fish matrix just prior to homogenization.

In the experiments on exposure to FFA and FM together, the initial extraction buffer was made 0.04% in FM, and FFA was added just prior to homogenization.

The proteins of isolated myofibriis and the salt-soluble proteins were separated by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis as previously described (Childs 1973a, c).

Effects of FFA on salt-soluble proteins — The amount of extractable salt-soluble protein decreased slightly following exposure to FFA (Fig. 1). SDS-acrylamide gel electrophoresis of the extracted salt-soluble proteins (Fig. 2) indicated that tropomyosin was the most readily insolubilized protein and was absent from the gel at 1% FFA. Myosin also appeared to be insolubilized, but this was a somewhat subjective observation since quantification of the protein content of acrylamide gels on other than a present–absent basis is difficult (Fishbein 1972).

Effect of FFA on whole myofibril — The whole myofibrils were much more sensitive to FFA than were their component proteins (Fig. 1). Regression analysis of the amount of isolable myofibrils (×) vs. FFA concentration (y) yielded the equation

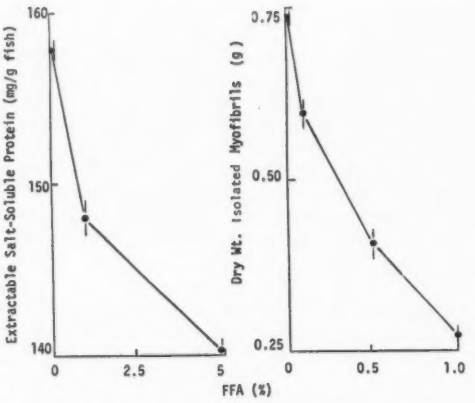

FIG. 1. Effects of Free fatty acids (safflower oil hydrolysate) on extractability of salt-soluble proteins and isolability of whole myofibrils from white muscle (8 g) of fresh lingcod (*Ophiodon elongatus*). All points are means of duplicate measurements of four replicates ± standard error of mean.

$$y = -6.82 \times -4.32$$

the correlation coefficient being -0.918. Electrophoresis of the isolated whole myofibrils indicated that they had complete protein complements (Fig. 2).

Combined effects of FFA and FM — The presence of small amounts of FM with FFA caused a further decrease in the amounts of extractable protein and isolable myofibrils (Fig. 3). The presence of 0.04% FM caused an approximate three-fold increase in both the insolubilization of salt-soluble proteins and the loss in suspensibility of whole myofibrils. Electrophoresis (Fig. 2) indicated that myosin and tropomyosin were the most readily insolubilized of the salt-soluble proteins.

Discussion — The experimental results demonstrated that the whole myofibril unit was more sensitive to FFA-induced loss in suspensibility than its constituent proteins were to insolubilization (decreases in isolable whole myofibrils and salt-soluble proteins are both observed as lack of suspensibility in appropriate buffer). This is consistent with earlier observations for FM alone and also for in situ frozen storage (Childs, 1973b, c). In addition, this study has shown that the extent of insolubilization of a single protein (tropomyosin) parallels the extent of loss in suspensibility of the whole myofibril. This also agrees with earlier studies with FM where the rate of loss in myofibril isolability paralleled the rate of decrease of myosin and tro-

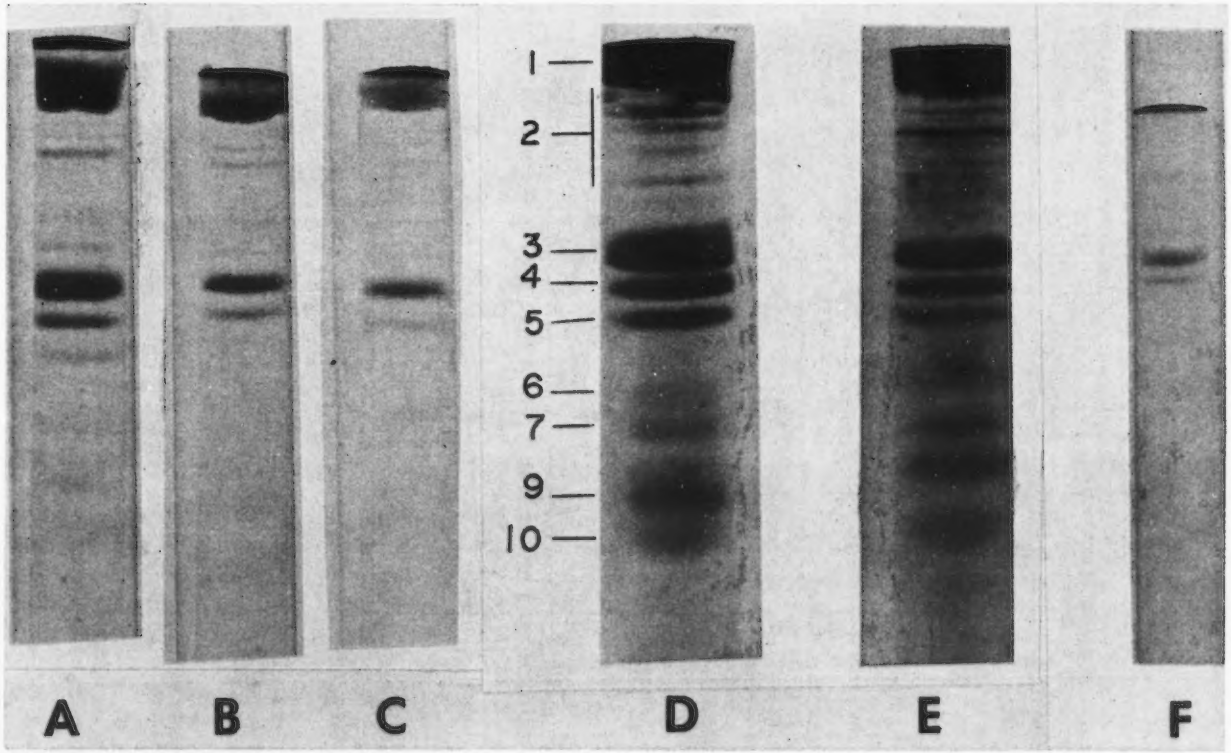

FIG. 2. SDS-acrylamide gel electrophoregrams of salt-soluble proteins and whole myofibrils from white muscle of fresh lingcod (cathode at bottom). A-C, of salt-soluble proteins after exposure to 0, 1, and 5% FFA, respectively. D, E, of isolated myofibrils before and after exposure to 5% FFA. F, of extracted salt-soluble proteins after exposure to 1% FFA and 0.04% formaldehyde. Identities of the salt-soluble proteins are suggested to be (Childs 1973a): 1, heavy chains of myosin; 2, actinins?; 3, actin; 4, 37,400 M.W. component; 5, tropomyosin; 6, 24,500 M.W. chain of myosin; 7, inhibitory factor; 9, 18,000 M.W. chain of myosin; 10, 15,000 M.W. chain of myosin.

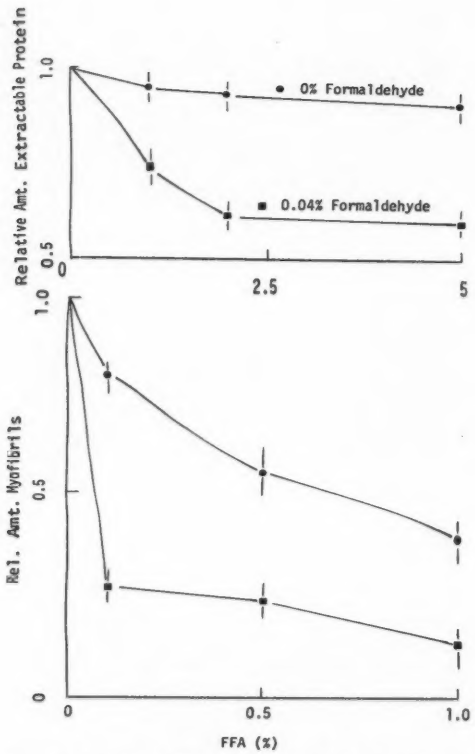

FIG. 3. Effects of FFA and formaldehyde in concert on extractability of salt-soluble proteins and isolability of whole myofibrils from white muscle of fresh lingcod. All points are means of duplicate measurements of four replicates ± standard error of mean.

pomyosin suspensibility (Childs 1973c). Therefore, evidence is accumulating that the whole myofibril unit may be more sensitive to deteriorative conditions associated with frozen storage than individual myofibrillar proteins.

The observed additonal effects of FM and FFA in concert as compared to FFA alone confirm earlier suggestions by Castell (1971) that small amounts of FM increase the activity of FFA. The chemical, physical, and/or biochemical basis for this is not readily apparent.

To prove that the whole myofibril is the main site of deterioration in frozen fish muscle, it will be necessary to analyze insoluble protein to determine if all myofibrillar species are present. If they are, apparently the myofibril loses suspensibility as a single unit, and protein insolubilization proceeds myofibril by myofibril. If not, it would appear that protein species are insolubilized on an individual basis.

Acknowledgments — This study was supported by the National Oceanic and Atmospheric Administration (maintained by the U.S. Department of Commerce). Institutional Sea Grant 04-3-158-4. Appreciation is expressed to J. Gaffke and M. DeBlois for their technical assistance. Gratitude is also extended to D. L. Crawford for his administrative assistance.

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A Method of Lipid Extraction Suitable for Use With an Electron Capture Detector

R. E. HURST

Department of the Environment
Fisheries and Marine Service, Vancouver Laboratory, Vancouver 8, B.C.

HURST, R. E. 1974. A method of lipid extraction for use with an electron capture detector. J. Fish. Res. Board Can. 31: 113–116.

A method of extracting oil is described in which ether is used as the lipophylic solvent in a monophase system. The method is as effective as the Bligh and Dyer method of extraction and avoids the use of chloroform, which interferes when gas chromatography is used with electron capture detection for analysis of the volatiles.

HURST, R. E. 1974. A method of lipid extraction for use with an electron capture detector. J. Fish. Res. Board Can. 31: 113–116.

L'auteur décrit une méthode d'extraction de l'huile selon laquelle l'éther est utilisé comme solvant lipophyle dans un système monophasé. Cette méthode est aussi efficace que la méthode d'extraction de Bligh et Dyer et évite l'emploi du chloroforme, qui produit de l'interférence quand on utilise la chromatographie en phase gazeuse et le détecteur à capture d'électrons dans l'analyse des substances volatiles.

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Gas-chromatographic analysis of headspace volatiles over oil can be very informative in following the development of rancidity. To obtain a more complete chromatographic profile, the effluent from the column can by split and directed into two different detectors, such as a flame ionization and electron capture (Bonelli 1967). However, in using this technique it was found that extraction of lipids with chloroform, as used for example in the Bligh and Dyer (1959) method, was not satisfactory due to the high degree of sensitivity shown to halogenated compounds by the electron capture detector.

In attempting to develop a suitable method of extraction the principle used in the Bligh and Dyer method was employed, namely to create a monophase system comprising the residual water of the tissue and methanol, but another lipophilic solvent was substituted in place of chloroform. Ether was chosen because it is a good lipid solvent, has a high vapor pressure, and is therefore an easy solvent to remove, and is not highly sensitive to electron capture detection. To determine the efficiency of such a monophase system employing ether, comparisons were made with the Bligh and Dyer method by extracting oil from the same substrate.

It was determined experimentally that water, ether, and methanol form a single phase in the proportions of 1:2:1.2, respectively. Solvents used in this work were ACS reagent grade which were distilled and examined for purity by gas chromatography.

Herring was chosen as a substrate and two similar fillets were taken from each fish, one to be extracted by the Bligh and Dyer method and the other by the ether monophase system. Trials were run by simultaneously extracting a single fish by both methods to ensure the greatest degree of uniformity. Water content of the fillets were determined to be 76±1.5% and the proper proportions of solvents calculated on this value.

Extraction — The procedure for the ether monophase extraction is as follows: a fillet is homogenized in an Omnimixer for 2 min with the appropriate volumes of ether and methanol, the homogenate left to stand 5 min and then vacuum filtered through Whatman No. 1 filter paper on a Pyrex Buchner funnel. The residual cake is washed with 50 ml ether–methanol 2:1 v/v followed by 50 ml of ether. The filtrate is transferred to a separatory funnel, 50 ml of water saturated with sodium chloride is added, and the resulting two phase system is allowed to equilibrate for 30 min. The ether layer is then withdrawn and dried over anhydrous sodium sulfate, and the solvent removed by vacuum flash distillation until a constant weight of oil is achieved.

Extraction by the Bligh and Dyer method was carried out as prescribed to the point of obtaining the filtrate. Then, to coincide with the method being tested, the residual cake was washed with 100 ml of chloroform and the filtrate transferred to a separatory funnel and left to equilibrate for 30 min. The chloroform layer was then isolated and dried over anhydrous sodium sulfate, and the solvent removed by flash evaporation.

Further, the aqueous methanol phase from both the established and proposed methods was reextracted with *n*-hexane to determine the oil content. Similarly the residual cakes were treated with hydrochloric acid and boiled, and the lipid material extracted in accord with the AOAC procedure (Official Methods of Analysis 1965).

Tests — Weights of oil at each step were determined. One gram aliquots of oil obtained by the two methods were titrated with .25 N KOH to compare their acidities, and thin-layer chromatography was used to determine if any qualitative differences were apparent. Finally, oil obtained by both methods was dissolved in *n*-heptane to indicate any nonlipid residues.

TABLE 1. Comparison of the Bligh and Dyer method and the monophase system of extracting lipids.

Trial	Method	Wt of tissue (g)	Wt of oil extracted (g)	Wt of oil (g) left in		% total oil extracted	Total FFA μmoles/g oil
				Aqueous	Residue		
1	Ether	61	11.44	.05	.06	99.04	50
	$CHCl_3$		11.52	.09	.05	98.79	52
2	Ether	35	5.90	.04	.07	98.16	
	$CHCl_3$		6.65	.02	.04	99.10	
3	Ether	41	7.25	.02	.05	99.31	50
	$CHCl_3$		6.74	.04	.04	98.82	50
4	Ether	41	7.58	.03	.06	98.82	
	$CHCl_3$		7.64	.02	.05	99.09	
5	Ether	14	2.17	.02	.02	98.19	152
	$CHCl_3$		2.28	.01	.03	98.27	158
Mean	Ether					98.7 (S.D. 0.51)	
	$CHCl_3$					98.8 (S.D. 0.34)	

Gas-chromatographic analysis — Headspace of the oil extracted by each procedure was monitored by gas chromatography during the process of solvent removal. To prevent the ether-extracted oil from being contaminated by chloroform, two flash evaporators were used, one for each solvent type and operated under identical conditions of temperature and vacuum. Headspace sampling was carried out at 15-min intervals by disconnecting the round bottom flask containing the oil from the evaporator, placing aluminium foil over the neck of the flask to act as a septum and withdrawing 0.5 ml of headspace with a Hamilton gas-tight syringe. The sample was injected onto a Micro-Tek MT-220 chromatograph equipped with an effluent splitter and two detectors, flame ionization and electron capture. A 12 ft × 3/16 inch OD aluminum column packed with 10% DEGA/10% CARBOWAX 20M on Chromosorb P and operating at a temperature of 80 C was used. The carrier gas was nitrogen with a flow rate of 40 ml/min.

Results and discussion — The percent of the total oil extracted by the monophase ether system compared very favorably with that for the Bligh and Dyer method (Table 1), as indicated by the calculated mean values of 98.7 and 98.8%, respectively.

Thin-layer chromatographic comparisons of oils extracted by the two methods revealed no visible differences in composition (Fig. 1). Further, there

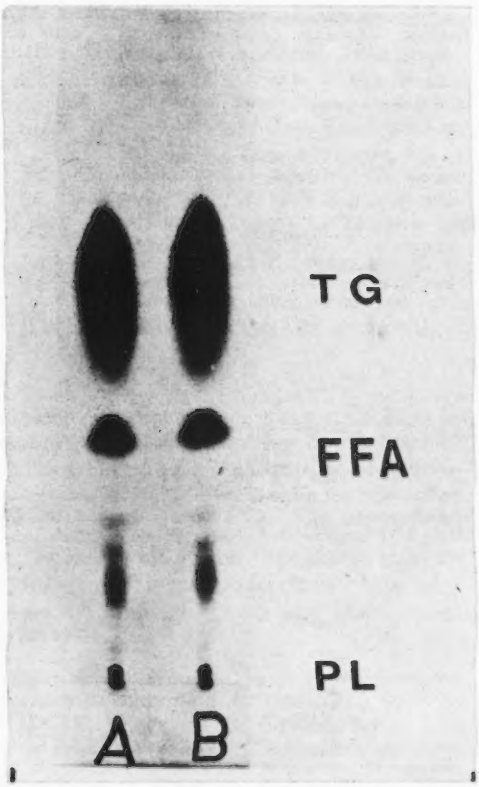

FIG. 1. Thin layer chromatograms of oil extracted by the ether monophase system (A) and the Bligh and Dyer method (B) TG, triglycerides; FFA, free fatty acids; PL, phospholipids. Adsorbent: Silica Gel G. Solvent: Hexane, diethyl ether, glacial acetic acid (90:10:1, v/v/v). Indicator:charring with sulfuric acid solution.

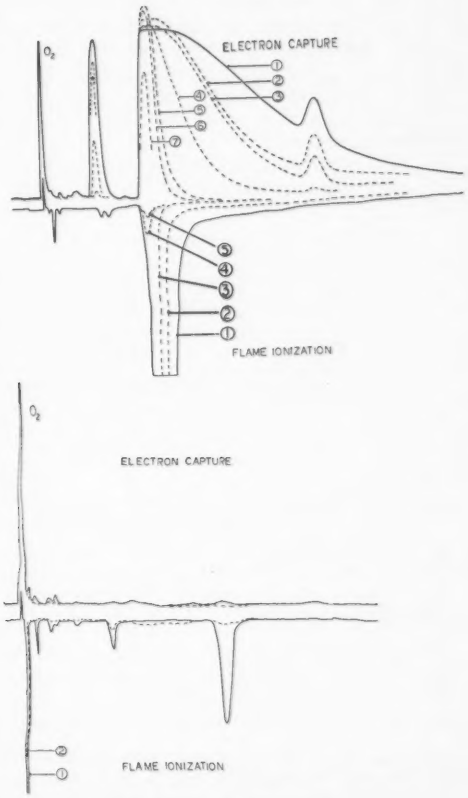

FIG. 2. Gas chromatograph of headspace from herring oil using dual channel flame ionization and electron capture detection. (*above*) Extraction by the Bligh and Dyer method. Solid line (1) residual chloroform after 15 min flash evaporation. Broken lines: 2, 30 min; 3, 45 min; 4, 1 hr; 5, 1 hr 15 min; 6, 1 hr 30 min; 7, 1 hr 45 min. (below) Extraction by ether monophase system. Solid line (1) residual ether after 15 min. Broken line (2) 30 min.

was no indication of nonlipid material present when the oils were dissolved in *n*-heptane.

Titration of the oil indicated that the two procedures are equally efficient in the extraction of acids. The means, each calculated on the basis of four titrations, give a fatty acid value of 79.0 μmoles/g of oil for the ether monophase and 79.2 μmoles/g for the Bligh and Dyer method.

There was a marked difference in the time necessary to remove the two solvents however. Flash evaporation extended for $1\frac{1}{2}$ hr was not sufficient to remove chloroform below the level of detection by electron capture (Fig. 2). Ether, on the other hand, was effectively removed by the same process in 15 min (Fig. 3). The two other peaks associated with the chloroform (Fig. 2) are present in headspace from reagent grade chloroform but are absent after redistillation. However, when the redistilled chloroform is used for extraction the two peaks reappear during flash evaporation.

The results of the monophase ether system compare very favorably with those of the Bligh and Dyer method. There is, however, no indication that it is in any way inherently a better method, except for work in which chloroform is an undesirable solvent or in which all traces of solvent must be removed quickly from the extract. Ether used in the described procedure must be carefully distilled to ensure no peroxides are present that could initiate autoxidation, particularly where autoxidation of the lipid is the subject of study.

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Production and Movement of Juvenile Rainbow Trout (*Salmo gairdneri*) in a Headwater of Bothwell's Creek, Georgian Bay, Canada

D. Ross Alexander[1] and Hugh R. MacCrimmon

Department of Zoology, University of Guelph, Guelph, Ont.

Alexander, D. R., and H. R. MacCrimmon. 1974. Production and movement of juvenile rainbow trout (*Salmo gairdneri*) in a headwater of Bothwell's Creek, Georgian Bay, Canada. J. Fish. Res. Board Can. 31: 117–121.

Within the 21,500 m² headwater, the standing population of juvenile rainbow trout reached a high of 7.05 g/m² in October. Production was maximum during August at 1.77 g/m². Total annual production is calculated at 284.5 kg (13.2 g/m²). Spring emigrants (no less than 4830 fish weighing 69 kg) were age I (91%) and age II. Minimum calculated ratio of production to yield as emigrants was 4.1:1. Comparatively few age 0 fish emigrated during summer. Because of their demonstrated capability to produce juveniles, sensitive headwaters must be preserved from ecological disturbance to assure self-perpetuating rainbow trout populations in the Great Lakes.

Alexander, D. R., and H. R. MacCrimmon. 1974. Production and movement of juvenile rainbow trout (*Salmo gairdneri*) in a headwater of Bothwell's Creek, Georgian Bay, Canada. J. Fish. Res. Board Can. 31: 117–121.

Sur une superficie de 21,500 m² de la tête des eaux, la biomasse des jeunes truites arc-en-ciel (*Salmo gairdneri*) atteint un sommet de 7.05 g/m² en octobre. Le maximum de production, 1.77 g/m², se produit en août. La production annuelle totale est calculée à 284.5 kg (13.2 g/m²). Les émigrants de printemps (pas moins de 4830 poissons pesant 69 kg) sont d'âge I (91%) et II. Le rapport minimal calculé production:rendement comme émigrants est de 4.1:1. Relativement peu de poissons d'âge 0 émigrent en été. Parce qu'elles s'avèrent capables de produire des jeunes truites et que par ailleurs elles sont sensibles, les têtes des cours d'eau doivent être protégées contre les dérangements écologiques, afin d'assurer aux Grands lacs des populations continues de truites arc-en-ciel.

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Although natural reproduction of anadromous rainbow trout (*Salmo gairdneri*) in tributaries of the Great Lakes sustains substantial sport fisheries, there is negligible published data on the production and migration of juvenile fish. This paper documents findings on juvenile rainbow trout in Bothwell's Creek, a tributary of Lake Huron, in which details on the spawning population have been previously published (Dodge and MacCrimmon 1970, 1971).

Materials and methods—The study was conducted in an 8.6 km headwater of Bothwell's Creek (43°30′N; 80°45′W), a tributary of the Georgian Bay of Lake Huron during 1969 and 1970. The headwater, averaging 2.5 m in width and 16 m/km in gradient, is supplied continuously by groundwater keeping the water cool in summer and ice-free in winter.

Movements of juvenile trout between the headwater and downstream were monitored by a modified Shetterstrap (Shetter 1938). Electro-weld screening (0.4 or 0.8 mesh/cm) was used in trap and leads except during the summer months when regalvanized hardware cloth was necessary to retain young-of-the-year trout. Water temperature and stream discharge was recorded daily. Scale samples were collected from all but young-of-the-year migrants and growth data taken from all fish captured.

Population estimates of trout resident in the headwater were made at three representative sampling stations, each 50 m long with an approx area of 255.2 m². Collections were made with a portable electric shocker (MacCrimmon and Berst 1963) and population estimates made by the removal method (Zippin 1958) in each of October and November of 1969; and January, March, May, August, September, and November of 1970. Confidence limits were not applicable because of the small numbers of fish (less than 50) in some age groups. Population estimates and mean weights were calculated for the beginning of each month.

[1]Present address: Department of the Environment, Fisheries and Marine Service, P.O. Box 550, Halifax, N.S.

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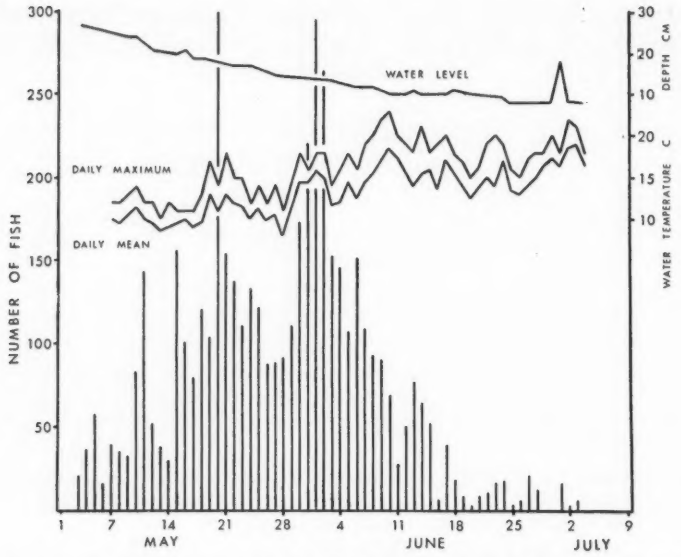

FIG. 1. Headwater emigration of juvenile rainbow trout through a trap on Bothwell's Creek during May and June, 1970.

Headwater production (Allen 1969) was calculated as the product of the daily instantaneous growth rate and the mean biomass present on the first day of consecutive months (Chapman 1967; Ricker 1946).

Results — (*Seasonal movement*) — Intermittent trap operation from the fall of 1969 to the spring of 1970 indicated the emigration of few resident trout prior to April 7 when trapping was discontinued because of severe flooding. Between May 2 and July 8, 4830 emigrant trout were captured (Fig. 1). Daily emigration rates tended to increase with rising water temperatures, while stream discharge decreased from 1.53 to 0.23 m^3/sec.

One-year-old fish (10.6 $\pm$ 0.5 cm) comprised 91%, two-year-old fish (14.9 $\pm$ 0.7 cm) 8.8% and three-year-old fish less than 0.2% of the total number of emigrants. Early emigrants (May 3–14) were larger (mean of 12.1 $\pm$ 0.6 cm) than later emigrants (June 7–14) with a mean length of only 10.4 $\pm$ 0.6 cm. No upstream movement of migrants through the trap was recorded during the winter or spring months. Emigration occurred principally at night. For example, during an hourly count over 24 hr on May 14 and 15, 141 fish emigrated between sunset and sunrise, but only five fish emigrated during the day.

From a length/weight regression (log weight = $\bar{2}$.008 + 2.995 log length), total weight of the 4830 fish caught while emigrating between May 2 and July 8 was calculated at 69, 369 g. This is a minimum estimate as some fish undoubtedly moved downstream prior to installation of the trap.

Summer movements of trout through the trap were limited principally to age 0 fish (Table 1). During summer trapping in 1969, only 11 older juveniles left the headwaters and 0 immigrated into it. In 1970, the numbers were 9 and 12, respectively.

TABLE 1. Movements of young-of-the-year rainbow trout through trap at low limit of Bothwell's Creek headwater.

		1969		1970	
		No. of fish	No. of intervals	No. of fish	No. of intervals
Upstream	day	51	17	25	24
	night	55	21	10	25
	total	106		35	
	day	299	17	81	24
	night	753[a]	21	179[a]	25
	total	1052[a]		260[a]	

[a]Numbers minimal because of probable escapement of fish during heavy freshets.

TABLE 2. Population estimates (N) and biomass estimates (B) for the 1968, 1969, and 1970 year-classes of rainbow trout resident within three collection sites (data combined) in Bothwell's Creek on the dates indicated. Growth rate per day (G), mean biomass ($\overline{B}$), and production (P) has been calculated for the intervals between dates (no./m² and wt./m² in parentheses).

Date	Year-class	N	B g	Year-class	G × 10^{-3}	$\overline{B}$ g	P g
Nov. 1/69	1968	21(0.08)	451.5(1.77)				
	1969	263(1.03)	1025.7(4.02)				
				1968	3.67	441.8(1.73)	48.6(0.19)
				1969	1.67	912.6(3.58)	45.6(0.18)
Dec. 1/69	1968	18(0.07)	432.0(1.69)				
	1969	195(0.76)	799.5(3.13)				
				1968	–3.55	398.8(1.63)	–43.9(–0.17)
				1969	1.54	801.8(3.14)	38.2(0.15)
Jan. 1/70	1968	17(0.07)	365.5(1.43)				
	1969	187(0.73)	804.1(3.15)				
				1968	–1.54	346.8(1.36)	–16.5(–0.06)
				1969	1.47	822.8(3.22)	37.4(0.15)
Feb. 1/70	1968	16(0.06)	328.0(1.29)				
	1969	187(0.73)	841.5(3.30)				
				1968	1.70	325.3(1.27)	15.5(0.06)
				1969	1.55	855.5(3.35)	37.2(0.15)
Mar. 1/70	1968	15(0.06)	322.5(1.26)				
	1969	185(0.72)	869.5(3.41)				
				1968	2.87	314.0(1.23)	27.9(0.11)
				1969	6.22	939.2(3.68)	181.2(0.71)
Apr. 1/70	1968	13(0.05)	305.5(1.20)				
	1969	177(0.69)	1008.9(3.95)				
				1968	5.24	304.0(1.19)	47.8(0.19)
				1969	11.71	1164.6(4.56)	409.2(1.60)
May 1/70	1968	11(0.04)	302.5(1.19)				
	1969	163(0.64)	1320.3(5.17)				
				1968	9.57	262.3(1.03)	77.8(0.30)
				1969	8.68	1269.7(4.98)	341.5(1.34)
June 1/70	1968	6(0.02)	222.0(0.87)				
	1969	115(0.45)	1219.0(4.78)				
June 15/70	1970	700(2.74)	105.0(0.41)				
				1968	8.68	183.0(0.72)	47.1(0.19)
				1969	6.80	1064.5(4.17)	217.3(0.85)
				1970	58.10	179.0(0.70)	166.4(0.65)
July 1/70	1968	3(0.01)	144.0(0.56)				
	1969	70(0.27)	910.0(3.57)				
	1970	666(2.61)	253.1(0.99)				
				1968	6.66	131.0(0.51)	27.0(0.16)
				1969	5.67	865.8(3.39)	152.3(0.60)
				1970	28.87	399.5(1.57)	357.6(1.40)
Aug. 1/70	1968	2(0.01)	118.0(0.46)				
	1969	53(0.21)	821.5(3.22)				
	1970	587(2.30)	545.9(2.14)				
				1968	0	88.5(0.35)	0
				1969	4.28	844.4(3.31)	112.1(0.44)
				1970	21.30	584.3(2.29)	451.9(1.77)
Sept. 1/70	1968	1(0.00)	59.0(0.23)				
	1969	49(0.19)	867.3(3.40)				
	1970	457(1.79)	822.6(3.22)				
				1968	–	–	–
				1969	1.65	822.2(3.34)	42.5(0.17)
				1970	14.13	892.6(3.50)	378.3(1.48)

(*Continued*)

TABLE 2. Population estimates (N) and biomass estimates (B) for the 1968, 1969, and 1970 year-classes of rainbow trout resident within three collection sites (data combined) in Bothwell's Creek on the dates indicated. Growth rate per day (G), mean biomass ($\overline{B}$), and production (P) has been calculated for the intervals between dates (no./m² and wt./m² in parentheses). — *(Concluded)*

Date	Year-class	N	B g	Year-class	G × 10^{-3}	$\overline{B}$ g	P g
Oct. 1/70	1968	0	0				
	1969	45(0.18)	837.0(3.28)				
	1970	350(1.37)	962.5(3.77)				
				1968	–	–	–
				1969	0	790.5(3.10)	0
				1970	5.88	976.3(3.83)	178.0(0.70)
Nov. 1/70	1968	0	0				
	1969	40(0.16)	744.0(2.92)				
	1970	300(1.18)	990.0(3.88)				
Total				1968			231.3(0.91)
				1969			1614.5(6.33)
				1970			1532.2(6.00)
Grand Total							3378.0(13.24)

Movement of all age 0 juveniles captured in 1969 and 1970 was predominately downstream (93%) and occurred largely at night. Large increases in stream discharge resulted in above-average emigrations of juveniles during both day and night periods. Upstream movement was greater during the daytime in 1970 and spread about equally between day and night in 1969. Summer water temperatures ranged from 12 to 23 C in 1969 and from 13 to 23 C in 1970.

Headwater population and production — Daily instantaneous growth rate of the 1968 year-class fell to a minimum of -3.55×10^{-3} in December of 1969 and subsequently increased to a maximum of 9.57×10^{-3} during the following May (Table 2). The growth rate then declined gradually through July, reaching a negligible level in August 1970. Standing crop declined after reaching a maximum of 1.77 g/m² on November 1, 1969, disappearing from the headwater by the autumn of 1970.

Daily instantaneous growth rate of the 1969 year-class remained stable at about 1.5×10^{-3} from November 1969 to February 1970, reaching a low of 1.47×10^{-3} during January (Table 2). Growth rate increased sharply to 11.71×10^{-3} in April but gradually declined thereafter, becoming negligible by October. Production followed a similar trend, reaching a maximum of 1.6 g/m² during April. The standing crop (5.17 g/m²) was greatest on May 1 and declined to a low of 2.92 g/m² by November 1, 1970.

The 1970 year-class became identifiable as free-swimming fry in mid-June. Daily growth rate declined from a high of 58.1×10^{-3} in June to 5.88×10^{-3} in October (Table 2). Production increased from 0.65 g/m² in June to 1.77 g/m² in August and 0.70 g/m² in October. Standing crop increased gradually from the time of emergence to reach a maximum of 3.88 g/m² in November.

Production of the three year-classes of juvenile rainbow trout resident in the sampling stations over a 12-month period extending from November 1,

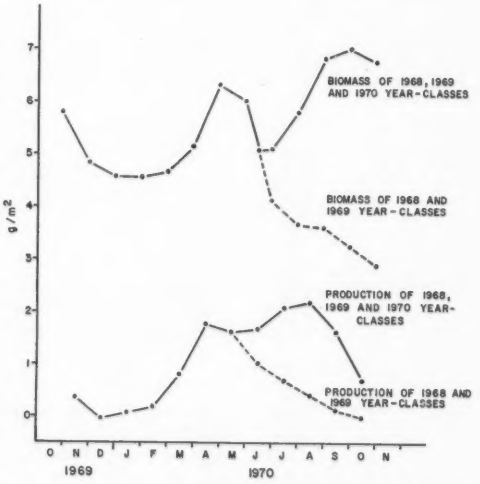
BIOMASS OF 1968, 1969 AND 1970 YEAR-CLASSES
BIOMASS OF 1968 AND 1969 YEAR-CLASSES
PRODUCTION OF 1968, 1969 AND 1970 YEAR-CLASSES
PRODUCTION OF 1968 AND 1969 YEAR-CLASSES
g/m²
0 1 2 3 4 5 6 7
O N D J F M A M J J A S O N
1969
1970

FIG. 2. Biomass and production of rainbow trout resident within three headwater stations on Bothwell's Creek.

1969 to November 1, 1970 is estimated at 3378 g, or 13.24 g/m^2 (Table 2). Only 19% of this weight was contributed by fish older than one year. Assuming that the three sample stations were representative of the headwater, total production was no less than 284.6 kg, the ratio of production to yield being 4.1:1. Combined production of the 1968 and 1969 year-classes reached a peak in April 1970 and biomass began to decline before recruitment of the 1970 year-class (Fig. 2).

Discussion — Three facts became apparent from an analysis of emigration data and statistics gathered on the resident headwaters population of juvenile rainbow trout in Bothwell's Creek.

Firstly, spring emigrants were principally age I juveniles (91%) with the remainder being age II fish. This ratio varies from the findings of Dodge and MacCrimmon (1970) that nearly all adults returning to Bothwell's Creek in previous years had a juvenile history of two years of stream life. One explanation is that the headwater habitat is no longer favoring the residency of trout older than age I because of a changing ecosystem on the upper watershed. Other plausible explanations are that there is a prolonged residency of age I emigrants in downstream habitats, that there is a comparatively high mortality of juveniles emigrating before age II, or a combination of these two phenomena. In any case, the discrepancy serves to demonstrate the need for the examination of smolt runs rather than inferring the age structure of emigrating juveniles from an analysis of scales of returning adults.

Secondly, summer emigrants were principally young-of-the-year fish and, in comparison to the numbers remaining in the headwaters (estimated at 56,100 and 49,500 fish on July 1 and August 1, 1970), amounted to comparatively few fish. The prior spring exodus of age I fish left vacant much habitat for summer occupation by age 0 trout. The headwaters of Bothwell's Creek is considered to be a highly fertile rearing ground for many age I and comparatively few age II juvenile rainbow trout. It is because of similar headwaters in other Great Lakes tributaries that the long established naturalized population has thrived and provided substantial sport fisheries in the Great Lakes basin (MacCrimmon and Gots 1972).

Thirdly, a comparison of the calculated headwater production (13.2 g/m^2) and the ratio of production to yield (4.1:1) for emigrating juveniles confirms that this water ranks high among salmonid streams in relative production level (Allen 1951; Chapman 1965; Goodnight and Bjornn 1971; LeCren 1969). In view of the capacity of the headwater of Bothwell's Creek, and presumably those of a number of other Great Lakes tributaries, to produce large quantities of juvenile emigrants resulting from natural production, it is imperative that these sensitive headwater environments be protected from ecological disturbances associated with land use change if self-sustaining populations of rainbow trout are to be preserved in Canadian waters of Lake Huron and the lower Great Lakes.

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Sex Ratios and Lengths of Adult Sea Lampreys (*Petromyzon marinus*) from a Lake Ontario Tributary

I. C. POTTER

School of Biological Sciences, University of Bath
Claverton Down, Bath BA2 7AY, Somerset, U.K.

F. W. H. BEAMISH

Department of Zoology, University of Guelph, Guelph, Ont.

AND B. G. H. JOHNSON

Environment Canada Sea Lamprey Control Unit
Ship Canal Post Office Box, Sault Ste. Marie, Ont.

POTTER, I. C., F. W. H. BEAMISH, AND B. G. H. JOHNSON. 1974. Sex ratios and lengths of adult sea lampreys (*Petromyzon marinus*) from a Lake Ontario tributary. J. Fish Res. Board Can. 31: 122–124.

The mean lengths of adult males and females of the sea lamprey (*Petromyzon marinus*) migrating into the Humber River from Lake Ontario in each of the years 1968–1972 varied little, and were similar to those found by other workers in recently established populations in the upper lakes. In contrast, the ratio of males to females, which lay within the narrow range of 1:1 to 1.26:1, was similar to those reported for long-established populations.

POTTER, I. C., F. W. H. BEAMISH, AND B. G. H. JOHNSON. 1974. Sex ratios and lengths of adult sea lampreys (*Petromyzon marinus*) from a Lake Ontario tributary. J. Fish Res. Board Can. 31: 122–124.

La longueur moyenne des lamproies marines (*Petromyzon marinus*) adultes mâles et femelles qui ont émigré dans la rivière Humber en provenance du lac Ontario durant chacune des années 1968–1972 n'a que peu varié et était semblable à celles observées par d'autres chercheurs chez des populations récemment établies dans les lacs supérieurs. Par contre, le rapport mâles:femelles, compris dans l'étroite gamme de 1:1 à 1.26:1, était semblable à ceux signalés chez des populations déjà établies depuis longtemps.

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THIS is an account of the lengths and sex ratios of adult sea lampreys (*Petromyzon marinus*) collected at a dam on the Humber River about 5 km from Lake Ontario, during there upstream migration in the years 1968 to 1972. The data suggest that the lamprey population in the lake may have been relatively stable in the 1960s.

A characteristic of the period when the sea lamprey, *Petromyzon marinus*, was spreading rapidly through lakes Huron, Michigan, and Superior, was the trend towards an increasing preponderance of males in the upstream migrant populations of successive years (Smith 1971). While a preponderance of males is apparently typical of long-established populations of various lampreys (Dean and Sumner 1898; Wigley 1959; Abakumov 1960; Zanandrea 1961; Hardisty and Potter 1971), the detailed studies of Hardisty (1961) on the non-parasitic species *Lampetra planeri* have shown that the percentage of adult males (although always greater than that of females) tends to oscillate, a high relative number of males being almost invariably associated with larger spawning populations. During the marked decline phase in the upper lakes, due to treatment of the streams with a selective larvicide (Applegate et al. 1961) and presumably also to a reduction in host density, *P. marinus* has shown a reversal towards an excess of females with the percentage of adult males in Lake Superior, for example, declining from 69.6 in 1963 to 26.7 in 1969 (Smith 1971). Another characteristic of the sea lamprey in the upper lakes over the last 20–25 years has been the gradual decline in the body length of spawning-run migrants (Smith 1971).

Since *P. marinus* has been present in Lake Ontario from at least 1835, and possibly even since the end of the Pleistocene (Lark 1972), samples from a Lake Ontario tributary provide an interesting com-

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parison with records from the lakes above the Welland Canal, where the lampreys have only been established during the last 40 or so years (Smith 1971). Furthermore, while the larvicide (TFM) has been used in the tributaries of the upper lakes for over a decade, it was not introduced into the rivers of Lake Ontario until 1971 (J. J. Tibbles, Sea Lamprey Control Unit, Sault Ste Marie, personal communication).

Since ammocoetes are not caught in the Humber River, the dam must prevent upstream migrants from reaching satisfactory spawning sites. Thus, the samples from this river are probably representative of Lake Ontario tributaries as they were derived from individuals which were not homing to their natal streams. Moreover, individuals in samples from the 5 years were derived from larvae that had not been subjected to TFM and, since they were collected from within a few kilometers of the river mouth, they had almost certainly migrated recently from the lake.

The length data (Fig. 1) show between years a close correspondence in mean length, in modal value, and in the pattern of length distribution. Furthermore, these data are similar to recent records from the upper lakes (Smith 1971), and to those

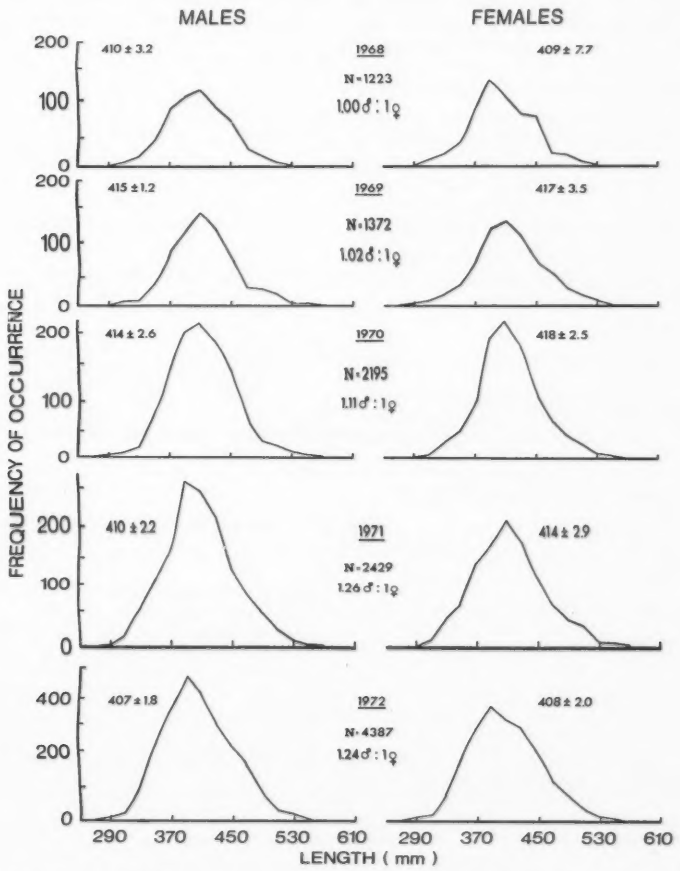

FIG. 1. Length-frequency curves, mean lengths (± 2 standard errors), and sex ratios for upstream migrating *Petromyzon marinus* caught in the Humber River, Lake Ontario, between 1968 and 1972. Measurements were made on animals preserved in 10% formalin. N = size of sample.

from long-established populations in Lake Cayuga (Wigley 1958).

In contrast, the sex ratios differed from those recorded over the last few years in the upper lakes, where the females were in a pronounced excess (Smith 1971; J. J. Tibbles personal communication). The ratio of males to females for the 5 years lay in the remarkably narrow range of parity to 1.26:1.

The small variation in the sex ratios for the different years, but with a marked tendency towards a small excess of males, as in long-established populations of other species of lampreys, together with the homogeneity in the length data, suggests that the lamprey population of Lake Ontario was relatively stable during the 1960s. Such a situation would not be surprising when one considers the relative antiquity of the sea lamprey in this region, and its highly destructive ability, as these features would have necessitated attainment of some degree of stability between the host and predator populations. In fact, although features such as either construction of dams or over-fishing (Christie 1972), might have influenced the relative abundance of teleosts at certain times during the last 100 or so years, subsequent oscillations in the lamprey populations would almost certainly have been less pronounced than those in the upper lakes, where the lamprey is of recent origin and has been subjected to TFM treatment.

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CARSON, W. V. 1973. Chemical conditions in the northwest Miramichi River during 1972. Fish. Res. Board Can. MS. Rep. 1254: 30 p. (6)

BUTLER, T. H., A. N. YATES, AND C. C. WOOD. 1973. *G. B. Reed* shrimp cruise 73-S-1, May 7–23, 1973. Fish. Res. Board Can. MS. Rep. 1255: 46 p. (1)

BERNARD, F. R., AND D. C. MILLER. 1973. Morphometric data for a preliminary investigation on the red sea urchin resources of British Columbia (*Strongylocentrotus franciscanus* Ag.). Fish. Res. Board Can. MS. Rep. 1256: 97 p. (1)

CARSON, W. G. 1973. The levels of nitrogen, oxygen, and humic acid in the dechlorinated freshwater system at the biological station determined and monitored over a period of one year. Fish. Res. Board Can. MS. Rep. 1257: 18 p. (6)

FORRESTER, C. R., AND M. S. SMITH. 1973. Species composition of rockfish in catches by Canadian commercial and research vessels in Queen Charlotte Sound. Fish. Res. Board Can. MS. Rep. 1258: 11 p. (1)

WESTRHEIM, S. J., AND W. R. HARLING. 1973. Report on the 1972 comparison of Pacific ocean perch otolith and scale interpretations. Fish. Res. Board Can. MS. Rep. 1259: 24 p. (1)

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

HEALY, M. C. 1974. Experimental cropping of lakes: II. Physical and chemical features of the lakes. Fish. Res. Board Can. Tech. Rep. 384: (In press) (4)

MCDANIEL, N. G. 1973. A survey of the benthic macroinvertebrate fauna and pollutants in Howe Sound. Fish. Res. Board Can. Tech. Rep. 385: 64 p. (3)

KELSO, J. R. M. 1974. Movement of yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieui*) and white bass (*Morone chrysops*) released in Long Point Bay, Lake Erie, during 1971 and 1972. Fish. Res. Board Can. Tech. Rep. 386: (In press) (11)

MUNDIE, J. H., D. E. MOUNCE, AND L. E. SMITH. 1973. Observations on the response of zoobenthos to additions of hay, willow leaves, alder leaves and cereal grain to stream substrates. Fish. Res. Board Can. Tech. Rep. 387: 123 p. (1)

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SCARSBROOK, J. R., AND J. MCDONALD. 1973. Purse seine catches of sockeye salmon (*Oncorhynchus nerka*) and other species of fish at Babine Lake, British Columbia, 1972. Fish. Res. Board Can. Tech. Rep. 390: 46 p. (1)

WICKETT, W. P., AND J. A. THOMSON. 1973. Transport computations and Ekmon transport charts for the North Pacific Ocean, 1972 (with a note on vorticity). Fish. Res. Board Can. Tech. Rep. 391: 133 p. (1)

LEAR, W. H. 1973. Size and age composition of the 1971 Newfoundland Labrador commercial salmon catch. Fish. Res. Board Can. Tech. Rep. 392: 43 p. (9)

LEVINGS, C. D. 1973. Sediments and abundance of *Lycodopsis pacifica* (Pisces, Zoarcidae) near Point Grey, B.C., with catch data for associated demersal fish. Fish. Res. Board Can. Tech. Rep. 393: 15 p. (3)

LESLIE, J., L. DEVEY, M. MUNAVAR, A. NAUWERCK, AND E. PICKETT. 1974. A comparison of electronic particle counters and inverted microscope methods for counting phytoplankton. Fish. Res. Board Can. Tech. Rep. 394: (In press) (11)

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KNIGHT, W., AND A. V. TYLER. 1973. Are species association coefficients really necessary? Fish. Res. Board Can. Tech. Rep. 397: 8 p. (6)

SMITH, J. E. 1973. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1972. Fish. Res. Board Can. Tech. Rep. 398: 83 p. (1)

HOURSTON, A. S., AND F. W. NASH. 1973. Computer programs for population analysis of B.C. herring from catch, sampling and spawn deposition data. Fish. Res. Board Can. Tech. Rep. 399: 334 p. (1)

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assemblages on the southwest Scotian Shelf. Fish. Res. Board Can. Tech. Rep. 402: 18 p. (6)

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Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. A limited number of copies are available for distribution as long as the supply lasts, or may be obtained by interlibrary loan, from the station where the translation has been deposited. Information on translations generally may be obtained from the Office of the Editor.

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BRUMBERG, V. A., AND L. Z. PEVZNER. 1972. Isoenzymes in nerve tissue. Usp. Sovrem. Biol. 74: 213–230. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2738, 1973) (8)

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REIMOLD, W. V., AND K. LANG. 1970. Effect of substituting coconut fat for a fish oil with high polyunsaturated acid content on the serum lipids of rats in a feeding experiment of long duration. Z. Ernahrungseiss. 10: 145–152. (Transl. from German by Fish. Res. Board Can. Transl. Ser. No. 2747, 1973) (8)

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REICHENBACH-KLINKE, H. 1966. The blood picture of fishes; its relationship to diseases and the influence of sewage poisons. Bull. Off. Int. Epizool. 65: 52–58. (Transl. from German by Fish. Res. Board Can. Transl. Ser. No. 2749, 1973) (8)

KARIUS, V. G., V. N. DEGTYAREV, AND V. V. KUZNETSOVA. 1971. On photoelectric sorting of flatfish into size groups. (Mechanization and automation of labor-consuming operations in fish processing). Tr. Vses. Nauchno—Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. (VNIRO) 78: 85–89. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2750, 1973) (2)

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Additional Publications

The Board also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements de l'Office situés à Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être éxécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).

Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter O and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

BULLETIN 177

L'intoxication paralysante par les mollusques dans l'Est du Canada

A. Prakash ★ J. C. Medcof ★ A. D. Tennant

Environment Canada
Fisheries and Marine Service

Environnement Canada
Service des pêches et des sciences de la mer

Traduction française du bulletin de A. Prakash, J. C. Medcof et A. D. Tennant intitulé « Paralytic shellfish poisoning in eastern Canada, » publié en 1971. Cet ouvrage rassemble tous les renseignements receuillés jusqu'à maintenant sur le problème de l'intoxication paralysante par les mollusques dans l'Est du Canada.

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